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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
C12N 15/90, 15/85, C12Q 1/68, C12N 5/10, 9/12, 15/13, C07K 16/28, C12N 15/12, C07K 14/705, G01N 33/53, C12N 15/62, C07K 19/00

A1

(11) International Publication Number: WO 98/41645

(43) International Publication Date: 24 September 1998 (24.09.98)

(21) International Application Number: PCT/US98/03935

(22) International Filing Date: 9 March 1998 (09.03.98)

(30) Priority Data:

08/819,866	14 March 1997 (14.03.97)	US
09/023,715	13 February 1998 (13.02.98)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

(57) Abstract

A method for achieving site specific integration of a desired DNA at a target site in a mammalian cell via homologous recombination is described. This method provides for the reproducible selection of cell lines wherein a desired DNA is integrated at a predetermined transcriptionally active site previously marked with a marker plasmid. The method is particularly suitable for the production of mammalian cell lines which secrete mammalian proteins at high levels, in particular immunoglobulins. Vectors and vector combinations for use in the subject cloning method are also provided.

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Title of the Invention

METHOD FOR INTEGRATING GENES AT SPECIFIC SITES IN MAMMALIAN CELLS VIA HOMOLOGOUS RECOMBINATION AND VECTORS FOR ACCOMPLISHING THE SAME

Field of the Invention

The present invention relates to a process of targeting the integration of a desired exogenous DNA to a specific location within the genome of a mammalian cell. More specifically, the invention describes a novel method for identifying a transcriptionally active target site ("hot spot") in the mammalian genome, and inserting a desired DNA at this site via homologous recombination. The invention also optionally provides the ability for gene amplification of the desired DNA at this location by co-integrating an amplifiable selectable marker, e.g., DHFR, in combination with the exogenous DNA. The invention additionally describes the construction of novel vectors suitable for accomplishing the above, and further provides mammalian cell lines produced by such methods which contain a desired exogenous DNA integrated at a target hot spot.

Background

Technology for expressing recombinant proteins in both prokaryotic and eukaryotic organisms is well established. Mammalian cells offer significant advantages over bacteria or yeast for protein production, resulting from their ability to correctly assemble, glycosylate and post-translationally modify recombinantly expressed proteins. After transfection into the host cells, recombinant expression constructs can be maintained as extrachromosomal elements, or may be integrated into the host cell genome. Generation of stably transfected mammalian cell lines usually involves the latter; a DNA construct encoding a gene of interest along with a drug resistance gene (dominant selectable marker) is introduced into the host cell, and subsequent growth in the presence of the drug allows for the selection of cells that have successfully integrated the exogenous DNA. In many instances, the gene of interest is linked to a drug resistant selectable marker which can later be subjected to gene amplification. The gene encoding dihydrofolate reductase (DHFR) is most commonly used for this purpose. Growth of cells in the presence of methotrexate, a competitive inhibitor of DHFR, leads to increased DHFR production by means of amplification of the DHFR gene. As flanking regions of DNA will also become amplified, the resultant coamplification of a DHFR linked gene in the transfected cell line can lead to increased protein

production, thereby resulting in high level expression of the gene of interest.

While this approach has proven successful, there are a number of problems with the system because of the random nature of the integration event. These problems exist because expression levels are greatly influenced by the effects of the local genetic environment at the gene locus, a phenomena well documented in the literature and generally referred to as "position effects" (for example, see Al-Shawi et al, *Mol. Cell. Biol.*, 10:1192-1198 (1990); Yoshimura et al, *Mol. Cell. Biol.*, 7:1296-1299 (1987)). As the vast majority of mammalian DNA is in a transcriptionally inactive state, random integration methods offer no control over the transcriptional fate of the integrated DNA. Consequently, wide variations in the expression level of integrated genes can occur, depending on the site of integration. For example, integration of exogenous DNA into inactive, or transcriptionally "silent" regions of the genome will result in little or no expression. By contrast integration into a transcriptionally active site may result in high expression.

Therefore, when the goal of the work is to obtain a high level of gene expression, as is typically the desired outcome of genetic engineering methods, it is generally necessary to screen large numbers of transfectants to find such a high producing clone.

Additionally, random integration of exogenous DNA into the genome can in some instances disrupt important cellular genes, resulting in an altered phenotype. These factors can make the generation of high expressing stable mammalian cell lines a complicated and laborious process.

Recently, our laboratory has described the use of DNA vectors containing translationally impaired dominant selectable markers in mammalian gene expression. (This is disclosed in U.S. Serial No. 08/147,696 filed November 3, 1993, recently allowed).

These vectors contain a translationally impaired neomycin phosphotransferase (neo) gene as the dominant selectable marker, artificially engineered to contain an intron into which a DHFR gene along with a gene or genes of interest is inserted. Use of these vectors as expression constructs has been found to significantly reduce the total number of drug resistant colonies produced, thereby facilitating the screening procedure in relation to conventional mammalian expression vectors. Furthermore, a significant percentage of the clones obtained using this system are high expressing clones. These results are apparently attributable to the modifications made to the neo selectable marker. Due to the translational impairment of the neo gene, transfected cells will not produce enough neo protein to survive drug selection, thereby decreasing the overall

number of drug resistant colonies. Additionally, a higher percentage of the surviving clones will contain the expression vector integrated into sites in the genome where basal transcription levels are high, resulting in overproduction of neo, thereby allowing the cells to overcome the impairment of the neo gene. Concomitantly, the genes of interest linked to neo will be subject to similar elevated levels of transcription. This same advantage is also true as a result of the artificial intron created within neo; survival is dependent on the synthesis of a functional neo gene, which is in turn dependent on correct and efficient splicing of the neo introns. Moreover, these criteria are more likely to be met if the vector DNA has integrated into a region which is already highly transcriptionally active.

Following integration of the vector into a transcriptionally active region, gene amplification is performed by selection for the DHFR gene. Using this system, it has been possible to obtain clones selected using low levels of methotrexate (50nM), containing few (<10) copies of the vector which secrete high levels of protein (>55pg/cell/day). Furthermore, this can be achieved in a relatively short period of time. However, the success in amplification is variable. Some transcriptionally active sites cannot be amplified and

therefore the frequency and extent of amplification from a particular site is not predictable.

Overall, the use of these translationally impaired vectors represents a significant improvement over other methods of random integration. However, as discussed, the problem of lack of control over the integration site remains a significant concern.

One approach to overcome the problems of random integration is by means of gene targeting, whereby the exogenous DNA is directed to a specific locus within the host genome. The exogenous DNA is inserted by means of homologous recombination occurring between sequences of DNA in the expression vector and the corresponding homologous sequence in the genome. However, while this type of recombination occurs at a high frequency naturally in yeast and other fungal organisms, in higher eukaryotic organisms it is an extremely rare event. In mammalian cells, the frequency of homologous versus non-homologous (random integration) recombination is reported to range from 1/100 to 1/5000 (for example, see Capecchi, *Science*, 244:1288-1292 (1989); Morrow and Kucherlapati, *Curr. Op. Biotech.*, 4:577-582 (1993)).

One of the earliest reports describing homologous recombination in mammalian cells comprised an artificial system created in mouse fibroblasts (Thomas et al, *Cell*, 44:419-428 (1986)). A cell line containing a mutated, non-functional version of the neo gene integrated into

the host genome was created, and subsequently targeted with a second non-functional copy of neo containing a different mutation. Reconstruction of a functional neo gene could occur only by gene targeting. Homologous recombinants were identified by selecting for G418 resistant cells, and confirmed by analysis of genomic DNA isolated from the resistant clones.

Recently, the use of homologous recombination to replace the heavy and light immunoglobulin genes at endogenous loci in antibody secreting cells has been reported. (U.S. Patent No. 5,202,238, Fell et al, (1993).) However, this particular approach is not widely applicable, because it is limited to the production of immunoglobulins in cells which endogenously express immunoglobulins, e.g., B cells and myeloma cells. Also, expression is limited to single copy gene levels because co-amplification after homologous recombination is not included. The method is further complicated by the fact that two separate integration events are required to produce a functional immunoglobulin: one for the light chain gene followed by one for the heavy chain gene.

An additional example of this type of system has been reported in NS/0 cells, where recombinant immunoglobulins are expressed by homologous recombination into the immunoglobulin gamma 2A locus (Hollis et al, international patent application #

PCT/IB95 (00014).) Expression levels obtained from this site were extremely high - on the order of 20pg/cell/day from a single copy integrant. However, as in the above example, expression is limited to this level because an amplifiable gene is not contegrated in this system. Also, other researchers have reported aberrant glycosylation of recombinant proteins expressed in NS/0 cells (for example, see Flesher et al, *Biotech. and Bioeng.*, 48:399-407 (1995)), thereby limiting the applicability of this approach.

The cre-loxP recombination system from bacteriophage P1 has recently been adapted and used as a means of gene targeting in eukaryotic cells. Specifically, the site specific integration of exogenous DNA into the Chinese hamster ovary (CHO) cell genome using cre recombinase and a series of lox containing vectors have been described. (Fukushige and Sauer, *Proc. Natl. Acad. Sci. USA*, 89:7905-7909 (1992).) This system is attractive in that it provides for reproducible expression at the same chromosomal location. However, no effort was made to identify a chromosomal site from which gene expression is optimal, and as in the above example, expression is limited to single copy levels in this system. Also, it is complicated by the fact that one needs to provide for expression of a functional recombinase enzyme in the mammalian cell.

The use of homologous recombination between an introduced DNA sequence and its endogenous chromosomal locus has also been reported to provide a useful means of genetic manipulation in mammalian cells, as well as in yeast cells. (See e.g., Bradley et al, *Meth. Enzymol.*, 223:855-879 (1993); Capecchi, *Science*, 244:1288-1292 (1989); Rothstein et al, *Meth. Enzymol.*, 194:281-301 (1991)). To date, most mammalian gene targeting studies have been directed toward gene disruption ("knockout") or site-specific mutagenesis of selected target gene loci in mouse embryonic stem (ES) cells. The creation of these "knockout" mouse models has enabled scientists to examine specific structure-function issues and examine the biological importance of a myriad of mouse genes. This field of research also has important implications in terms of potential gene therapy applications.

Also, vectors have recently been reported by Celltech (Kent, U.K.) which purportedly are targeted to transcriptionally active sites in NSO cells, which do not require gene amplification (Peakman et al, *Hum. Antibod. Hybridomas*, 5:65-74 (1994)). However, levels of immunoglobulin secretion in these unamplified cells have not been reported to exceed 20pg/cell/day, while in amplified CHO cells, levels as high as 100pg/cell/day can be obtained (Id.).

It would be highly desirable to develop a gene targeting system which reproducibly provided for the integration of exogenous DNA into a predetermined site in the genome known to be transcriptionally active. Also, it would be desirable if such a gene targeting system would further facilitate co-amplification of the inserted DNA after integration. The design of such a system would allow for the reproducible and high level expression of any cloned gene of interest in a mammalian cell, and undoubtedly would be of significant interest to many researchers.

In this application, we provide a novel mammalian expression system, based on homologous recombination occurring between two artificial substrates contained in two different vectors. Specifically, this system uses a combination of two novel mammalian expression vectors, referred to as a "marking" vector and a "targeting" vector.

Essentially, the marking vector enables the identification and marking of a site in the mammalian genome which is transcriptionally active, i.e., a site at which gene expression levels are high. This site can be regarded as a "hot spot" in the genome. After integration of the marking vector, the subject expression system enables another DNA to be integrated at this site, i.e., the targeting vector, by means of homologous recombination occurring between DNA sequences common to

both vectors. This system affords significant advantages over other homologous recombination systems.

Unlike most other homologous systems employed in mammalian cells, this system exhibits no background. Therefore, cells which have only undergone random integration of the vector do not survive the selection. Thus, any gene of interest cloned into the targeting plasmid is expressed at high levels from the marked hot spot. Accordingly, the subject method of gene expression substantially or completely eliminates the problems inherent to systems of random integration, discussed in detail above. Moreover, this system provides reproducible and high level expression of any recombinant protein at the same transcriptionally active site in the mammalian genome. In addition, gene amplification may be effected at this particular transcriptionally active site by including an amplifiable dominant selectable marker (e.g. DHFR) as part of the marking vector.

<u>Objects of the Invention</u>

Thus, it is an object of the invention to provide an improved method for targeting a desired DNA to a specific site in a mammalian cell.

It is a more specific object of the invention to provide a novel method for targeting a desired DNA to a specific site in a mammalian cell via homologous recombination.

It is another specific object of the invention to provide novel vectors for achieving site specific integration of a desired DNA in a mammalian cell.

It is still another object of the invention to provide novel mammalian cell lines which contain a desired DNA integrated at a predetermined site which provides for high expression.

It is a more specific object of the invention to provide a novel method for achieving site specific integration of a desired DNA in a Chinese hamster ovary (CHO) cell.

It is another more specific object of the invention to provide a novel method for integrating immunoglobulin genes, or any other genes, in mammalian cells at predetermined chromosomal sites that provide for high expression.

It is another specific object of the invention to provide novel vectors and vector combinations suitable for integrating immunoglobulin genes into mammalian cells at predetermined sites that provide for high expression.

It is another object of the invention to provide mammalian cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression.

It is an even more specific object of the invention to provide a novel method for integrating immunoglobulin

genes into CHO cells that provide for high expression, as well as novel vectors and vector combinations that provide for such integration of immunoglobulin genes into CHO cells.

In addition, it is a specific object of the invention to provide novel CHO cell lines which contain immunoglobulin genes integrated at predetermined sites that provide for high expression, and have been amplified by methotrexate selection to secrete even greater amounts of functional immunoglobulins.

Brief Description of the Figures

Figure 1 depicts a map of a marking plasmid according to the invention referred to as Desmond. The plasmid is shown in circular form (1a) as well as a linearized version used for transfection (1b).

Figure 2(a) shows a map of a targeting plasmid referred to "Molly". Molly is shown here encoding the anti-CD20 immunoglobulin genes, expression of which is described in Example 1.

Figure 2(b) shows a linearized version of Molly, after digestion with the restriction enzymes *Kpn*1 and *Pac*1. This linearized form was used for transfection.

Figure 3 depicts the potential alignment between Desmond sequences integrated into the CHO genome, and incoming targeting Molly sequences. One potential ar-

rangement of Molly integrated into Desmond after homologous recombination is also presented.

Figure 4 shows a Southern analysis of single copy Desmond clones. Samples are as follows:

Lane 1: λHindIII DNA size marker

Lane 2: Desmond clone 10F3

Lane 3: Desmond clone 10C12

Lane 4: Desmond clone 15C9

Lane 5: Desmond clone 14B5

Lane 6: Desmond clone 9B2

Figure 5 shows a Northern analysis of single copy Desmond clones. Samples are as follows: Panel A: northern probed with CAD and DHFR probes, as indicated on the figure. Panel B: duplicate northern, probed with CAD and HisD probes, as indicated. The RNA samples loaded in panels A and B are as follows:

Lane 1: clone 9B2, lane 2; clone 10C12, lane 3; clone 14B5, lane 4; clone 15C9, lane 5; control RNA from CHO transfected with a HisD and DHFR containing plasmid, lane 6; untransfected CHO.

Figure 6 shows a Southern analysis of clones resulting from the homologous integration of Molly into Desmond. Samples are as follows:

Lane 1: λHindIII DNA size markers, Lane 2: 20F4, lane 3; 5F9, lane 4; 21C7, lane 5; 24G2, lane 6; 25E1, lane 7; 28C9, lane 8; 29F9, lane 9; 39G11, lane 10; 42F9, lane 11; 50G10, lane 12; Molly plasmid DNA, linearized with

BglII(top band) and cut with BglII and KpnI (lower band), lane 13; untransfected Desmond.

Figures 7A through 7G contain the Sequence Listing for Desmond.

Figures 8A through 8I contain the Sequence Listing for Molly-containing anti-CD20.

Figure 9 contains a map of the targeting plasmid, "Mandy," shown here encoding anti-CD23 genes, the expression of which is disclosed in Example 5.

Figures 10A through 10N contain the sequence listing of "Mandy" containing the anti-CD23 genes as disclosed in Example 5.

Detailed Description of the Invention

The invention provides a novel method for integrating a desired exogenous DNA at a target site within the genome of a mammalian cell via homologous recombination. Also, the invention provides novel vectors for achieving the site specific integration of a DNA at a target site in the genome of a mammalian cell.

More specifically, the subject cloning method provides for site specific integration of a desired DNA in a mammalian cell by transfection of such cell with a "marker plasmid" which contains a unique sequence that is foreign to the mammalian cell genome and which provides a substrate for homologous recombination, followed by transfection with a "target plasmid" containing

a sequence which provides for homologous recombination with the unique sequence contained in the marker plasmid, and further comprising a desired DNA that is to be integrated into the mammalian cell. Typically, the integrated DNA will encode a protein of interest, such as an immunoglobulin or other secreted mammalian glycoprotein.

The exemplified homologous recombination system uses the neomycin phosphotransferase gene as a dominant selectable marker. This particular marker was utilized based on the following previously published observations;

(i) the demonstrated ability to target and restore function to a mutated version of the neo gene (cited earlier) and

(ii) our development of translationally impaired expression vectors, in which the neo gene has been artificially created as two exons with a gene of interest inserted in the intervening intron; neo exons are correctly spliced and translated in vivo, producing a functional protein and thereby conferring G418 resistance on the resultant cell population. In this application, the neo gene is split into three exons. The third exon of neo is present on the "marker" plasmid and becomes integrated into the host cell genome upon integration of the marker plasmid into the mammalian cells. Exons 1 and 2 are present on the targeting plasmid, and are separated

by an intervening intron into which at least one gene of interest is cloned. Homologous recombination of the targeting vector with the integrated marking vector results in correct splicing of all three exons of the neo gene and thereby expression of a functional neo protein (as determined by selection for G418 resistant colonies). Prior to designing the current expression system, we had experimentally tested the functionality of such a triply spliced neo construct in mammalian cells. The results of this control experiment indicated that all three neo exons were properly spliced and therefore suggested the feasibility of the subject invention.

However, while the present invention is exemplified using the neo gene, and more specifically a triple split neo gene, the general methodology should be efficacious with other dominant selectable markers.

As discussed in greater detail *infra*, the present invention affords numerous advantages to conventional gene expression methods, including both random integration and gene targeting methods. Specifically, the subject invention provides a method which reproducibly allows for site-specific integration of a desired DNA into a transcriptionally active domain of a mammalian cell. Moreover, because the subject method introduces an artificial region of "homology" which acts as a unique substrate for homologous recombination and the

insertion of a desired DNA, the efficacy of subject invention does not require that the cell endogenously contain or express a specific DNA. Thus, the method is generically applicable to all mammalian cells, and can be used to express any type of recombinant protein.

The use of a triply spliced selectable marker, e.g., the exemplified triply spliced neo construct, guarantees that all G418 resistant colonies produced will arise from a homologous recombination event (random integrants will not produce a functional neo gene and consequently will not survive G418 selection). Thus, the subject invention makes it easy to screen for the desired homologous event. Furthermore, the frequency of additional random integrations in a cell that has undergone a homologous recombination event appears to be low.

Based on the foregoing, it is apparent that a significant advantage of the invention is that it substantially reduces the number of colonies that need be screened to identify high producer clones, i.e., cell lines containing a desired DNA which secrete the corresponding protein at high levels. On average, clones containing integrated desired DNA may be identified by screening about 5 to 20 colonies (compared to several thousand which must be screened when using standard random integration techniques, or several hundred using the previously described intronic insertion vectors) Additionally, as the site of integration was preselected

and comprises a transcriptionally active domain, all exogenous DNA expressed at this site should produce comparable, i.e. high levels of the protein of interest.

Moreover, the subject invention is further advantageous in that it enables an amplifiable gene to be inserted on integration of the marking vector. Thus, when a desired gene is targeted to this site via homologous recombination, the subject invention allows for expression of the gene to be further enhanced by gene amplification. In this regard, it has been reported in from the literature that different genomic sites have different capacities for gene amplification (Meinkoth et al, *Mol. Cell Biol.*, 7:1415-1424 (1987)). Therefore, this technique is further advantageous as it allows for the placement of a desired gene of interest at a specific site that is both transcriptionally active and easily amplified. Therefore, this should significantly reduce the amount of time required to isolate such high producers.

Specifically, while conventional methods for the construction of high expressing mammalian cell lines can take 6 to 9 months, the present invention allows for such clones to be isolated on average after only about 3-6 months. This is due to the fact that conventionally isolated clones typically must be subjected to at least three rounds of drug resistant gene amplification in order to reach satisfactory levels of gene expression.

As the homologously produced clones are generated from a preselected site which is a high expression site, fewer rounds of amplification should be required before reaching a satisfactory level of production.

Still further, the subject invention enables the reproducible selection of high producer clones wherein the vector is integrated at low copy number, typically single copy. This is advantageous as it enhances the stability of the clones and avoids other potential adverse side-effects associated with high copy number. As described *supra*, the subject homologous recombination system uses the combination of a "marker plasmid" and a "targeting plasmid" which are described in more detail below.

The "marker plasmid" which is used to mark and identify a transcriptionally hot spot will comprise at least the following sequences:

(i) a region of DNA that is heterologous or unique to the genome of the mammalian cell, which functions as a source of homology, allows for homologous recombination (with a DNA contained in a second target plasmid). More specifically, the unique region of DNA (i) will generally comprise a bacterial, viral, yeast synthetic, or other DNA which is not normally present in the mammalian cell genome and which further does not comprise significant homology or sequence identity to DNA contained in the genome of the mammalian cell.

Essentially, this sequence should be sufficiently different to mammalian DNA that it will not significantly recombine with the host cell genome via homologous recombination. The size of such unique DNA will generally be at least about 2 to 10 kilobases in size, or higher, more preferably at least about 10kb, as several other investigators have noted an increased frequency of targeted recombination as the size of the homology region is increased (Capecchi, *Science*, 244:1288-1292 (1989)).

The upper size limit of the unique DNA which acts as a site for homologous recombination with a sequence in the second target vector is largely dictated by potential stability constraints (if DNA is too large it may not be easily integrated into a chromosome and the difficulties in working with very large DNAs.

(ii) a DNA including a <u>fragment</u> of a selectable marker DNA, typically an exon of a dominant selectable marker gene. The only essential feature of this DNA is that it not encode a functional selectable marker protein unless it is expressed in association with a sequence contained in the target plasmid. Typically, the target plasmid will comprise the remaining exons of the dominant selectable marker gene (those not comprised in "targeting" plasmid). Essentially, a functional selectable marker should only be produced if homologous recombination occurs (resulting in the association and

expression of this marker DNA (i) sequence together with the portion(s) of the selectable marker DNA fragment which is (are) contained in the target plasmid).

As noted, the current invention exemplifies the use of the neomycin phosphotransferase gene as the dominant selectable marker which is "split" in the two vectors. However, other selectable markers should also be suitable, e.g., the Salmonella histidinol dehydrogenase gene, hygromycin phosphotransferase gene, herpes simplex virus thymidine kinase gene, adenosine deaminase gene, glutamine synthetase gene and hypoxanthine-guanine phosphoribosyl transferase gene.

(iii) a DNA which encodes a functional selectable marker protein, which selectable marker is different from the selectable marker DNA (ii). This selectable marker provides for the successful selection of mammalian cells wherein the marker plasmid is successfully integrated into the cellular DNA. More preferably, it is desirable that the marker plasmid comprise two such dominant selectable marker DNAs, situated at opposite ends of the vector. This is advantageous as it enables integrants to be selected using different selection agents and further enables cells which contain the entire vector to be selected. Additionally, one marker can be an amplifiable marker to facilitate gene amplification as discussed previously. Any of the

dominant selectable marker listed in (ii) can be used as well as others generally known in the art.

Moreover, the marker plasmid may optionally further comprise a rare endonuclease restriction site. This is potentially desirable as this may facilitate cleavage. If present, such rare restriction site should be situated close to the middle of the unique region that acts as a substrate for homologous recombination. Preferably such sequence will be at least about 12 nucleotides. The introduction of a double stranded break by similar methodology has been reported to enhance the frequency of homologous recombination. (Choulika et al, *Mol. Cell. Biol.*, 15:1968-1973 (1995)). However, the presence of such sequence is not essential.

The "targeting plasmid" will comprise at least the following sequences:

(1) the same unique region of DNA contained in the marker plasmid or one having sufficient homology or sequence identity therewith that said DNA is capable of combining via homologous recombination with the unique region (i) in the marker plasmid. Suitable types of DNAs are described *supra* in the description of the unique region of DNA (1) in the marker plasmid.

(2) The remaining exons of the dominant selectable marker, one exon of which is included as (ii) in the marker plasmid listed above. The essential features of this DNA fragment is that it result in a functional

(selectable) marker protein only if the target plasmid integrates via homologous recombination (wherein such recombination results in the association of this DNA with the other fragment of the selectable marker DNA contained in the marker plasmid) and further that it allow for insertion of a desired exogenous DNA. Typically, this DNA will comprise the remaining exons of the selectable marker DNA which are separated by an intron. For example, this DNA may comprise the first two exons of the neo gene and the marker plasmid may comprise the third exon (back third of neo).

(3) The target plasmid will also comprise a desired DNA, e.g., one encoding a desired polypeptide, preferably inserted within the selectable marker DNA fragment contained in the plasmid. Typically, the DNA will be inserted in an intron which is comprised between the exons of the selectable marker DNA. This ensures that the desired DNA is also integrated if homologous recombination of the target plasmid and the marker plasmid occurs. This intron may be naturally occurring or it may be engineered into the dominant selectable marker DNA fragment.

This DNA will encode any desired protein, preferably one having pharmaceutical or other desirable properties. Most typically the DNA will encode a mammalian protein, and in the current examples provided, an immunoglobulin or an immunoadhesin. However the

invention is not in any way limited to the production of immunoglobulins.

As discussed previously, the subject cloning method is suitable for any mammalian cell as it does not require for efficacy that any specific mammalian sequence or sequences be present. In general, such mammalian cells will comprise those typically used for protein expression, e.g., CHO cells, myeloma cells, COS cells, BHK cells, Sp2/0 cells, NIH 3T3 and HeLa cells. In the examples which follow, CHO cells were utilized. The advantages thereof include the availability of suitable growth medium, their ability to grow efficiently and to high density in culture, and their ability to express mammalian proteins such as immunoglobulins in biologically active form.

Further, CHO cells were selected in large part because of previous usage of such cells by the inventors for the expression of immunoglobulins (using the translationally impaired dominant selectable marker containing vectors described previously). Thus, the present laboratory has considerable experience in using such cells for expression. However, based on the examples which follow, it is reasonable to expect similar results will be obtained with other mammalian cells.

In general, transformation or transfection of mammalian cells according to the subject invention will be effected according to conventional methods. So that the

invention may be better understood, the construction of exemplary vectors and their usage in producing integrants is described in the examples below.

EXAMPLE 1

Design and Preparation of Marker and Targeting Plasmid DNA Vectors

The marker plasmid herein referred to as "Desmond" was assembled from the following DNA elements:

(a) Murine dihydrofolate reductase gene (DHFR), incorporated into a transcription cassette, comprising the mouse beta globin promoter 5" to the DHFR start site, and bovine growth hormone poly adenylation signal 3" to the stop codon. The DHFR transcriptional cassette was isolated from TCAE6, an expression vector created previously in this laboratory (Newman et al, 1992, *Biotechnology*, 10:1455-1460).

(b) *E. coli* ß-galactosidase gene - commercially available, obtained from Promega as pSV-b-galactosidase control vector, catalog # E1081.

(c) Baculovirus DNA, commercially available, purchased from Clontech as pBAKPAK8, cat # 6145-1.

(d) Cassette comprising promoter and enhancer elements from Cytomegalovirus and SV40 virus. The cassette was generated by PCR using a derivative of expression vector TCAE8 (Reff et al, *Blood*, 83:435-445 (1994)). The enhancer cassette was inserted within the baculo-

virus sequence, which was first modified by the insertion of a multiple cloning site.

(e) *E. coli* GUS (glucuronidase) gene, commercially available, purchased from Clontech as pB101, cat. # 6017-1.

(f) Firefly luciferase gene, commercially available, obtained from Promega as pGEM-Luc (catalog # E1541).

(g) S. typhimurium histidinol dehydrogenase gene (HisD). This gene was originally a gift from (Donahue et el, *Gene*, 18:47-59 (1982)), and has subsequently been incorporated into a transcription cassette comprising the mouse beta globin major promoter 5' to the gene, and the SV40 polyadenylation signal 3' to the gene.

The DNA elements described in (a)-(g) were combined into a pBR derived plasmid backbone to produce a 7.7kb contiguous stretch of DNA referred to in the attached figures as "homology". Homology in this sense refers to sequences of DNA which are not part of the mammalian genome and are used to promote homologous recombination between transfected plasmids sharing the same homology DNA sequences.

(h) Neomycin phosphotransferase gene from TN5 (Davis and Smith, *Ann. Rev. Micro.*, 32:469-518 (1978)). The complete neo gene was subcloned into pBluescript SK-(Stratagene catalog # 212205) to facilitate genetic manipulation. A synthetic linker was then inserted into

a unique Pst1 site occurring across the codons for amino acid 51 and 52 of neo. This linker encoded the necessary DNA elements to create an artificial splice donor site, intervening intron and splice acceptor site within the neo gene, thus creating two separate exons, presently referred to as neo exon 1 and 2. Neo exon 1 encodes the first 51 amino acids of neo, while exon 2 encodes the remaining 203 amino acids plus the stop codon of the protein A *Not*1 cloning site was also created within the intron.

Neo exon 2 was further subdivided to produce neo exons 2 and 3. This was achieved as follows: A set of PCR primers were designed to amplify a region of DNA encoding neo exon 1, intron and the first 111 2/3 amino acids of exon2. The 3' PCR primer resulted in the introduction of a new 5' splice site immediately after the second nucleotide of the codon for amino acid 111 in exon 2, therefore generating a new smaller exon 2. The DNA fragment now encoding the original exon 1, intron and new exon 2 was then subcloned and propagated in a pBR based vector. The remainder of the original exon 2 was used as a template for another round of PCR amplification, which generated "exon3". The 5' primer for this round of amplification introduced a new splice acceptor site at the 5' side of the newly created exon 3, i.e. before the final nucleotide of the codon for amino acid 111. The resultant 3 exons of neo encode the

following information: exon 1 - the first 51 amino acids of neo; exon 2 - the next 111 2/3 amino acids, and exon 3 the final 91 1/3 amino acids plus the translational stop codon of the neo gene.

Neo exon 3 was incorporated along with the above mentioned DNA elements into the marking plasmid "Desmond". Neo exons 1 and 2 were incorporated into the targeting plasmid "Molly". The *Not*1 cloning site created within the intron between exons 1 and 2 was used in subsequent cloning steps to insert genes of interest into the targeting plasmid.

A second targeting plasmid "Mandy" was also generated. This plasmid is almost identical to "Molly" (some restriction sites on the vector have been changed) except that the original HisD and DHFR genes contained in "Molly" were inactivated. These changes were incorporated because the Desmond cell line was no longer being cultured in the presence of Histidinol, therefore it seemed unnecessary to include a second copy of the HisD gene. Additionally, the DHFR gene was inactivated to ensure that only a single DHFR gene, namely the one present in the Desmond marked site, would be amplifiable in any resulting cell lines. "Mandy" was derived from "Molly" by the following modifications:

(i) A synthetic linker was inserted in the middle of the DHFR coding region. This linker created a stop codon and shifted the remainder of the DHFR coding

region out of frame, therefore rendering the gene nonfunctional.

(ii) A portion of the HisD gene was deleted and replaced with a PCR generated HisD fragment lacking the promoter and start codon of the gene.

Figure 1 depicts the arrangement of these DNA elements in the marker plasmid "Desmond". Figure 2 depicts the arrangement of these elements in the first targeting plasmid, "Molly". Figure 3 illustrates the possible arrangement in the CHO genome, of the various DNA elements after targeting and integration of Molly DNA into Desmond marked CHO cells. Figure 9 depicts the targeting plasmid "Mandy."

Construction of the marking and targeting plasmids from the above listed DNA elements was carried out following conventional cloning techniques (see, e.g., Molecular Cloning, A Laboratory Manual, J. Sambrook et al, 1987, Cold Spring Harbor Laboratory Press, and Current Protocols in Molecular Biology, F. M. Ausubel et al, eds., 1987, John Wiley and Sons). All plasmids were propagated and maintained in E. coli XLI blue (Stratagene, cat. # 200236). Large scale plasmid preparations were prepared using Promega Wizard Maxiprep DNA Purification System®, according to the manufacturer's directions.

EXAMPLE 2

Construction of a Marked CHO Cell Line

1. Cell Culture and Transfection Procedures to Produced Marked CHO Cell Line

Marker plasmid DNA was linearized by digestion overnight at 37°C with Bst1107I. Linearized vector was ethanol precipitated and resuspended in sterile TE to a concentration of 1mg/ml. Linearized vector was introduced into DHFR-Chinese hamster ovary cells (CHO cells) DG44 cells (Urlaub et al, *Som. Cell and Mol. Gen.*, 12:555-566 (1986)) by electroporation as follows.

Exponentially growing cells were harvested by centrifugation, washed once in ice cold SBS (sucrose buffered solution, 272mM sucrose, 7mM sodium phosphate, pH 7.4, 1mM magnesium chloride) then resuspended in SBS to a concentration of 10^7 cells/ml. After a 15 minute incubation on ice, 0.4ml of the cell suspension was mixed with 40μg linearized DNA in a disposable electroporation cuvette. Cells were shocked using a BTX electrocell manipulator (San Diego, CA) set at 230 volts, 400 microfaraday capacitance, 13 ohm resistance. Shocked cells were then mixed with 20 ml of prewarmed CHO growth media (CHO-S-SFMII, Gibco/BRL, catalog # 31033-012) and plated in 96 well tissue culture plates. Forty eight hours after electroporation, plates were fed with selection media (in the case of transfection with Desmond, selection media is CHO-S-SFMII without

hypoxanthine or thymidine, supplemented with 2mM Histidinol (Sigma catalog # H6647)). Plates were maintained in selection media for up to 30 days, or until some of the wells exhibited cell growth. These cells were then removed from the 96 well plates and expanded ultimately to 120 ml spinner flasks where they were maintained in selection media at all times.

EXAMPLE 3

Characterization of Marked CHO Cell Lines

(a) Southern Analysis

Genomic DNA was isolated from all stably growing Desmond marked CHO cells. DNA was isolated using the Invitrogen Easy® DNA kit, according to the manufacturer's directions. Genomic DNA was then digested with HindIII overnight at 37°C, and subjected to Southern analysis using a PCR generated digoxygenin labelled probe specific to the DHFR gene. Hybridizations and washes were carried out using Boehringer Mannheim's DIG easy hyb (catalog # 1603 558) and DIG Wash and Block Buffer Set (catalog # 1585 762) according to the manufacturer's directions. DNA samples containing a single band hybridizing to the DHFR probe were assumed to be Desmond clones arising from a single cell which had integrated a single copy of the plasmid. These clones were retained for further analysis. Out of a total of 45 HisD resistant cell lines isolated, only 5 were

single copy integrants. Figure 4 shows a Southern blot containing all 5 of these single copy Desmond clones. Clone names are provided in the figure legend.

(b) Northern Analysis

Total RNA was isolated from all single copy Desmond clones using TRIzol reagent (Gibco/BRL cat # 15596-026) according to the manufacturer's directions. 10-20μg RNA from each clone was analyzed on duplicate formaldehyde gels. The resulting blots were probed with PCR generated digoxygenin labelled DNA probes to (i) DHFR message, (ii) HisD message and (iii) CAD message. CAD is a trifunctional protein involved in uridine biosynthesis (Wahl et al, *J. Biol. Chem.*, 254, 17:8679-8689 (1979)), and is expressed equally in all cell types. It is used here as an internal control to help quantitate RNA loading. Hybridizations and washes were carried out using the above mentioned Boehringer Mannheim reagents. The results of the Northern analysis are shown in Figure 5. The single copy Desmond clone exhibiting the highest levels of both the His D and DHFR message is clone 15C9, shown in lane 4 in both panels of the figure. This clone was designated as the "marked cell line" and used in future targeting experiments in CHO, examples of which are presented in the following sections.

EXAMPLE 4

Expression of Anti-CD20 Antibody in Desmond Marked CHO Cells

C2B8, a chimeric antibody which recognizes B-cell surface antigen CD20, has been cloned and expressed previously in our laboratory. (Reff et al, *Blood*, 83:434-45 (1994)). A 4.1 kb DNA fragment comprising the C2B8 light and heavy chain genes, along with the necessary regulatory elements (eukaryotic promoter and polyadenylation signals) was inserted into the artificial intron created between exons 1 and 2 of the neo gene contained in a pBR derived cloning vector. This newly generated 5kb DNA fragment (comprising neo exon 1, C2B8 and neo exon 2) was excised and used to assemble the targeting plasmid Molly. The other DNA elements used in the construction of Molly are identical to those used to construct the marking plasmid Desmond, identified previously. A complete map of Molly is shown in Fig. 2.

The targeting vector Molly was linearized prior to transfection by digestion with *Kpn*1 and *Pac*1, ethanol precipitated and resuspended in sterile TE to a concentration of 1.5mg/mL. Linearized plasmid was introduced into exponentially growing Desmond marked cells essentially as described, except that 80μg DNA was used in each electroporation. Forty eight hours postelectroporation, 96 well plates were supplemented with selection medium - CHO-SSFMII supplemented with 400 μg/mL Geneti-

cin (G418, Gibco/BRL catalog # 10131-019). Plates were maintained in selection medium for up to 30 days, or until cell growth occurred in some of the wells. Such growth was assumed to be the result of clonal expansion of a single G418 resistant cell. The supernatants from all G418 resistant wells were assayed for C2B8 production by standard ELISA techniques, and all productive clones were eventually expanded to 120mL spinner flasks and further analyzed.

<u>Characterization of Antibody secreting Targeted Cells</u>

A total of 50 electroporations with Molly targeting plasmid were carried out in this experiment, each of which was plated into separate 96 well plates. A total of 10 viable, anti-CD20 antibody secreting clones were obtained and expanded to 120ml spinner flasks. Genomic DNA was isolated from all clones, and Southern analyses were subsequently performed to determine whether the clones represented single homologous recombination events or whether additional random integrations had occurred in the same cells. The methods for DNA isolation and Southern hybridization were as described in the previous section. Genomic DNA was digested with EcoRI and probed with a PCR generated digoxygenin labelled probe to a segment of the CD20 heavy chain constant region. The results of this Southern analysis are presented in figure 6. As can be seen in the figure, 8 of

the 10 clones show a single band hybridizing to the CD20 probe, indicating a single homologous recombination event has occurred in these cells. Two of the ten, clones 24G2 and 28C9, show the presence of additional band(s), indicative of an additional random integration elsewhere in the genome.

We examined the expression levels of anti-CD20 antibody in all ten of these clones, the data for which is shown in Table 1, below.

Table 1:

Expression Level of Anti-CD20 Secreting Homologous Integrants

Clone	Anti-CD20, pg/c/d
20F4	3.5
25E1	2.4
42F9	1.8
39G11	1.5
21C7	1.3
50G10	0.9
29F9	0.8
5F9	0.3
28C9*	4.5
24G2*	2.1

* These clones contained additional randomly integrated copies of anti-CD20. Expression levels of these clones therefore reflect a contribution from both the homologous and random sites.

Expression levels are reported as picogram per cell per day (pg/c/d) secreted by the individual clones, and represented the mean levels obtained from three separate ELISAs on samples taken from 120 mL spinner flasks.

As can be seen from the data, there is a variation in antibody secretion of approximately ten fold between the highest and lowest clones. This was somewhat unexpected as we anticipated similar expression levels from all clones due to the fact the anti-CD20 genes are all integrated into the same Desmond marked site. Nevertheless, this observed range in expression extremely small in comparison to that seen using any traditional random integration method or with our translationally impaired vector system.

Clone 20F4, the highest producing single copy integrant was selected for further study. Table 2 (below) presents ELISA and cell culture data from seven day production runs of this clone.

Table 2:

7 Day Production Run Data for 20F4

Day	% Viable	Viable/ml (x 10^5)	Tx2 (hr)	mg/L	pg/c/d
1	96	3.4	31	1.3	4.9
2	94	6	29	2.5	3.4
3	94	9.9	33	4.7	3.2
4	90	17.4	30	6.8	3
5	73	14		8.3	
6	17	3.5		9.5	

Clone 20F4 was seeded at 2×10^5ml in a 120ml spinner flask on day 0. On the following six days, cell counts were taken, doubling times calculated and 1ml samples of supernatant removed from the flask and analyzed for secreted anti-CD20 by ELISA.

This clone is secreting on average, 3-5pg antibody/-cell/day, based on this ELISA data. This is the same level as obtained from other high expressing single copy clones obtained previously in our laboratory using the previously developed translationally impaired random integration vectors. This result indicates the following:

(1) that the site in the CHO genome marked by the Desmond marking vector is highly transcriptionally active, and therefore represents an excellent site from which to express recombinant proteins, and

(2) that targeting by means of homologous recombination can be accomplished using the subject vectors and occurs at a frequency high enough to make this system a viable and desirable alternative to random integration methods.

To further demonstrate the efficacy of this system, we have also demonstrated that this site is amplifiable, resulting in even higher levels of gene expression and protein secretion. Amplification was achieved by plating serial dilutions of 20F4 cells, starting at a density of 2.5×10^4 cells/ml, in 96 well tissue culture dishes, and culturing these cells in media (CHO-SSFMII) supplemented with 5, 10, 15 or 20nM methotrexate. Antibody secreting clones were screened using standard ELISA techniques, and the highest producing clones were expanded and further analyzed. A summary of this amplification experiment is presented in Table 3 below.

Table 3:

Summary of 20F4 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
10	56	3-13	4	10-15
15	27	2-14	3	15-18
20	17	4-11	1	ND

Methotrexate amplification of 20F4 was set up as described in the text, using the concentrations of methotrexate indicated in the above table. Supernatants from all surviving 96 well colonies were assayed by ELISA, and the range of anti-CD20 expressed by these clones is indicated in column 3. Based on these results, the highest producing clones were expanded to 120ml spinners and several ELISAs conducted on the spinner supernatants to determine the pg/cell/day expression levels, reported in column 5.

The data here clearly demonstrates that this site can be amplified in the presence of methotrexate. Clones from the 10 and 15nM amplifications were found to produce on the order of 15-20pg/cell/day.

A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. A 15nM clone, designated 20F4-15A5, was selected as the highest expressing cell line. This clone originated

from a 96 well plate in which only 22 wells grew, and was therefore assumed to have arisen from a single cell. The clone was then subjected to a further round of methotrexate amplification. As described above, serial dilutions of the culture were plated into 96 well dishes and cultured in CHO-SS-FMII medium supplemented with 200, 300 or 400nM methotrexate. Surviving clones were screened by ELISA, and several high producing clones were expanded to spinner cultures and further analyzed. A summary of this second amplification experiment is presented in Table 4.

Table 4:

Summary of 20F4-15A5 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d, spinner
200	67	23-70	1	50-60
250	86	21-70	4	55-60
300	81	15-75	3	40-50

Methotrexate amplifications of 20F4-15A5 were set up and assayed as described in the text. The highest producing wells, the numbers of which are indicated in column 4, were expanded to 120ml spinner flasks. The expression levels of the cell lines derived from these wells is recorded as pg/c/d in column 5.

The highest producing clone came from the 250nM methotrexate amplification. The 250nM clone, 20F4-15A5-250A6 originated from a 96 well plate in which only wells

grew, and therefore is assumed to have arisen from a single cell. Taken together, the data in Tables 3 and 4 strongly indicates that two rounds of methotrexate amplification are sufficient to reach expression levels of 60pg/cell/day, which is approaching the maximum secretion capacity of immunoglobulin in mammalian cells (Reff, M.E., *Curr. Opin. Biotech.*, 4:573-576 (1993)). The ability to reach this secretion capacity with just two amplification steps further enhances the utility of this homologous recombination system. Typically, random integration methods require more than two amplification steps to reach this expression level and are generally less reliable in terms of the ease of amplification. Thus, the homologous system offers a more efficient and time saving method of achieving high level gene expression in mammalian cells.

EXAMPLE 5

Expression of Anti-Human CD23 Antibody in Desmond Marked CHO Cells

CD23 is low affinity IgE receptor which mediates binding of IgE to B and T lymphocytes (Sutton, B.J., and Gould, H.J., *Nature*, 366:421-428 (1993)). Anti-human CD23 monoclonal antibody 5E8 is a human gamma-1 monoclonal antibody recently cloned and expressed in our laboratory. This antibody is disclosed in commonly

assigned Serial No. 08/803,085, filed on February 20, 1997.

The heavy and light chain genes of 5E8 were cloned into the mammalian expression vector N5KG1, a derivative of the vector NEOSPLA (Barnett et al, in *Antibody Expression and Engineering*, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)) and two modifications were then made to the genes. We have recently observed somewhat higher secretion of immunoglobulin light chains compared to heavy chains in other expression constructs in the laboratory (Reff et al, 1997, unpublished observations). In an attempt to compensate for this deficit, we altered the 5E8 heavy chain gene by the addition of a stronger promoter/enhancer element immediately upstream of the start site. In subsequent steps, a 2.9kb DNA fragment comprising the 5E8 modified light and heavy chain genes was isolated from the N5KG1 vector and inserted into the targeting vector Mandy. Preparation of 5E8-containing Molly and electroporation into Desmond 15C9 CHO cells was essentially as described in the preceding section.

One modification to the previously described protocol was in the type of culture medium used. Desmond marked CHO cells were cultured in protein-free CD-CHO medium (Gibco-BRL, catalog # AS21206) supplemented with 3mg/L recombinant insulin (3mg/mL stock, Gibco-BRL, catalog # AS22057) and 8mM L-glutamine (200mM stock, Gibco-BRL, catalog # 25030-081). Subsequently, trans-

fected cells were selected in the above medium supplemented with 400μg/mL geneticin. In this experiment, 20 electroporations were performed and plated into 96 well tissue culture dishes. Cells grew and secreted anti-CD23 in a total of 68 wells, all of which were assumed to be clones originating from a single G418 cell. Twelve of these wells were expanded to 120ml spinner flasks for further analysis. We believe the increased number of clones isolated in this experiment (68 compared with 10 for anti-CD20 as described in Example 4) is due to a higher cloning efficiency and survival rate of cells grown in CD-CHO medium compared with CHO-SS-FMII medium. Expression levels for those clones analyzed in spinner culture ranged from 0.5-3pg/c/d, in close agreement with the levels seen for the anti-CD20 clones. The highest producing anti-CD23 clone, designated 4H12, was subjected to methotrexate amplification in order to increase its expression levels. This amplification was set up in a manner similar to that described for the anti-CD20 clone in Example 4. Serial dilutions of exponentially growing 4H12 cells were plated into 96 well tissue culture dishes and grown in CD-CHO medium supplemented with 3mg/L insulin, 8mM glutamine and 30, 35 or 40nM methotrexate. A summary of this amplification experiment is presented in Table 5.

Table 5:

Summary of 2H12 Amplification

nM MTX	# Wells Assayed	Expression Level mg/l 96 well	# Wells Expanded	Expression Level pg/c/d from spinner
30	100	6-24	8	10-25
35	64	4-27	2	10-15
40	96	4-20	1	ND

The highest expressing clone obtained was a 30nM clone, isolated from a plate on which 22 wells had grown. This clone, designated 4H12-30G5, was reproducibly secreting 18-22pg antibody per cell per day. This is the same range of expression seen for the first amplification of the anti CD20 clone 20F4 (clone 20F4-15A5 which produced 15-18pg/c/d, as described in Example 4). This data serves to further support the observation that amplification at this marked site in CHO is reproducible and efficient. A second amplification of this 30nM cell line is currently underway. It is anticipated that saturation levels of expression will be achievable for the anti-CD23 antibody in just two amplification steps, as was the case for anti-CD20.

EXAMPLE 6

Expression of Immunoadhesin in Desmond Marked CHO Cells

CTLA-4, a member of the Ig superfamily, is found on the surface of T lymphocytes and is thought to play a role in antigen-specific T-cell activation (Dariavach et al, *Eur. J. Immunol.*, 18:1901-1905 (1988); and Linsley et al, *J. Exp. Med.*, 174:561-569 (1991)). In order to further study the precise role of the CTLA-4 molecule in the activation pathway, a soluble fusion protein comprising the extracellular domain of CTLA-4 linked to a truncated form of the human IgG1 constant region was

created (Linsley et al (Id.). We have recently expressed this CTLA-4 Ig fusion protein in the mammalian expression vector BLECH1, a derivative of the plasmid NEOSPLA (Barnett et al, in Antibody Expression and Engineering, H.Y Yang and T. Imanaka, eds., pp27-40 (1995)). An 800bp fragment encoding the CTLA-4 Ig was isolated from this vector and inserted between the SacII and BglII sites in Molly.

Preparation of CTLA-4Ig-Molly and electroporation into Desmond clone 15C9 CHO cells was performed as described in the previous example relating to anti-CD20. Twenty electroporations were carried out, and plated into 96 well culture dishes as described previously. Eighteen CTLA-4 expressing wells were isolated from the 96 well plates and carried forward to the 120ml spinner stage. Southern analyses on genomic DNA isolated from each of these clones were then carried out to determine how many of the homologous clones contained additional random integrants. Genomic DNA was digested with BglII and probed with a PCR generated digoxygenin labelled probe to the human IgG1 constant region. The results of this analysis indicated that 85% of the CTLA-4 clones are homologous integrants only; the remaining 15% contained one additional random integrant. This result corroborates the findings from the expression of anti-CD20 discussed above, where 80% of the clones were single homologous integrants. Therefore, we can conclude

that this expression system reproducibly yields single targeted homologous integrants in at least 80% of all clones produced.

Expression levels for the homologous CTlA4-Ig clones ranged from 8-12pg/cell/day. This is somewhat higher than the range reported for anti-CD20 antibody and anti-CD23 antibody clones discussed above. However, we have previously observed that expression of this molecule using the intronic insertion vector system also resulted in significantly higher expression levels than are obtained for immunoglobulins. We are currently unable to provide an explanation for this observation.

EXAMPLE 7

Targeting Anti-CD20 to an alternate Desmond Marked CHO Cell Line

As we described in a preceding section, we obtained 5 single copy Desmond marked CHO cell lines (see Figures 4 and 5). In order to demonstrate that the success of our targeting strategy is not due to some unique property of Desmond clone 15C9 and limited only to this clone, we introduced anti-CD20 Molly into Desmond clone 9B2 (lane 6 in figure 4, lane 1 in figure 5). Preparation of Molly DNA and electroporation into Desmond 9B2 was exactly as described in the previous example pertaining to anti-CD20. We obtained one homologous integrant from this experiment. This clone was expanded to a 120ml

spinner flask, where it produced on average 1.2pg anti-CD20/cell/day. This is considerably lower expression than we observed with Molly targeted into Desmond 15C9. However, this was the anticipated result, based on our northern analysis of the Desmond clones. As can be seen in Figure 5, mRNA levels from clone 9B2 are considerably lower than those from 15C9, indicating the site in this clone is not as transcriptionally active as that in 15C9. Therefore, this experiment not only demonstrates the reproducibility of the system - presumably any marked Desmond site can be targeted with Molly - it also confirms the northern data that the site in Desmond 15C9 is the most transcriptionally active.

From the foregoing,.it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without diverting from the scope of the invention. Accordingly, the invention is not limited by the appended claims.

WHAT IS CLAIMED IS:

1. A method for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises the following steps:

(i) transfecting or transforming a mammalian cell with a first plasmid ("marker plasmid") containing the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid;

(ii) selecting a cell which contain the marker plasmid integrated in its genome;

(iii) transfecting or transforming said selected cell with a second plasmid ("target plasmid") which contains the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid; and

(iv) selecting cells which contain the target plasmid integrated at the target site by screening for the expression of the first selectable marker protein.

2. The method of Claim 1, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

3. The method of Claim 2, wherein the second plasmid contains the remaining exons of said first selectable marker.

4. The method of Claim 3, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

5. The method Claim 4, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in

the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

6. The method of Claim 3, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

7. The method of Claim 4, wherein the desired protein is a mammalian protein.

8. The method of Claim 7, wherein the protein is an immunoglobulin.

9. The method of Claim 1, which further comprises determining the RNA levels of the selectable marker (c) contained in the marker plasmid prior to integration of the target vector.

10. The method of Claim 9, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex

thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

11. The method of Claim 1, wherein the mammalian cell is selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

12. The method of Claim 11, wherein the cell is a CHO cell.

13. The method of Claim 1, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains the first two exons of the neomycin phosphotransferase gene.

14. The method of Claim 1, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

15. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

16. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is at least 300 nucleotides.

17. The method of Claim 16, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

18. The method of claim 17, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

19. The method of Claim 1, wherein the first selectable marker DNA is split into at least three exons.

20. The method of Claim 1, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

21. The method of Claim 20, wherein the unique region of DNA does not contain any functional genes.

22. A vector system for inserting a desired DNA at a target site in the genome of a mammalian cell which comprises at least the following:

(i) a first plasmid ("marker plasmid") containing at least the following sequences:

(a) a region of DNA that is heterologous to the mammalian cell genome which when integrated in the mammalian cell genome provides a unique site for homologous recombination;

(b) a DNA fragment encoding a portion of a first selectable marker protein; and

(c) at least one other selectable marker DNA that provides for selection of mammalian cells which have been successfully integrated with the marker plasmid; and

(ii) a second plasmid ("target plasmid") which contains at least the following sequences:

(a) a region of DNA that is identical or is sufficiently homologous to the unique region in the marker plasmid such that this region of DNA can recombine with said DNA via homologous recombination;

(b) a DNA fragment encoding a portion of the same selectable marker contained in the marker plasmid, wherein the active selectable marker protein encoded by said DNA is only produced if said fragment is expressed in association with the fragment of said selectable marker DNA contained in the marker plasmid.

23. The vector system of Claim 22, wherein the DNA fragment encoding a fragment of a first selectable marker is an exon of a dominant selectable marker.

24. The vector system of Claim 23, wherein the second plasmid contains the remaining exons of said first selectable marker.

25. The vector system of Claim 24, wherein at least one DNA encoding a desired protein is inserted between said exons of said first selectable marker contained in the target plasmid.

26. The vector system of Claim 24, wherein a DNA encoding a dominant selectable marker is further inserted between the exons of said first selectable marker contained in the target plasmid to provide for co-amplification of the DNA encoding the desired protein.

27. The vector system of Claim 24, wherein the first dominant selectable marker is selected from the group consisting of neomycin phosphotransferase, histidinol dehydrogenase, dihydrofolate reductase, hygromycin phosphotransferase, herpes simplex virus thymidine kinase, adenosine deaminase, glutamine synthetase, and hypoxanthine-guanine phosphoribosyl transferase.

28. The vector system of Claim 25, wherein the desired protein is a mammalian protein.

29. The vector system of Claim 28, wherein the protein is an immunoglobulin.

30. The vector system of Claim 22, wherein the other selectable marker contained in the marker plasmid is a dominant selectable marker selected from the group consisting of histidinol dehydrogenase, herpes simplex thymidine kinase, hydromycin phosphotransferase, adenosine deaminase and glutamine synthetase.

31. The vector system of Claim 22, which provides for insertion of a desired DNA at a targeted site in the genome of a mammalian cell selected from the group consisting of Chinese hamster ovary (CHO) cells, myeloma cells, baby hamster kidney cells, COS cells, NSO cells, HeLa cells and NIH 3T3 cells.

32. The vector system of Claim 31, wherein the mammalian cell is a CHO cell.

33. The vector system of Claim 22, wherein the marker plasmid contains the third exon of the neomycin phosphotransferase gene and the target plasmid contains

the first two exons of the neomycin phosphotransferase gene.

34. The vector system of Claim 22, wherein the marker plasmid further contains a rare restriction endonuclease sequence which is inserted within the region of homology.

35. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, a viral DNA or a synthetic DNA.

36. The vector system of Claim 22, wherein the unique region of DNA (a) contained in the marker plasmid vector system that provides for homologous recombination is at least 300 nucleotides.

37. The vector system of Claim 36, wherein the unique region of DNA ranges in size from about 300 nucleotides to 20 kilobases.

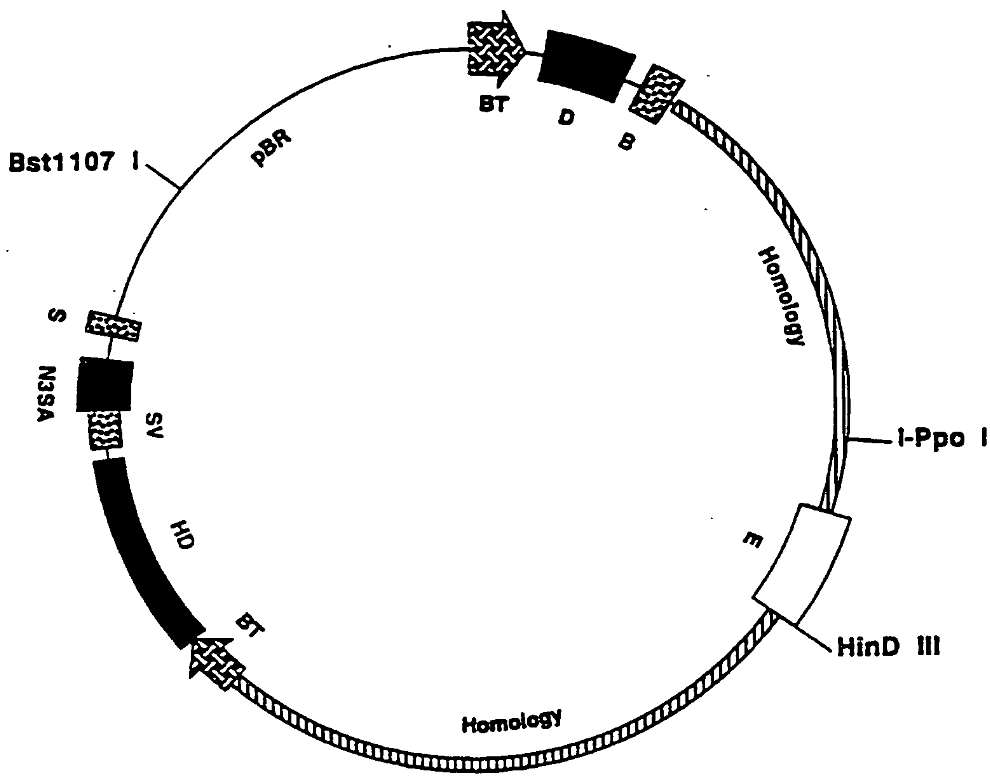
38. The vector system of Claim 37, wherein the unique region of DNA preferably ranges in size from 2 to 10 kilobases.

39. The vector system of Claim 22, wherein the first selectable marker DNA is split into at least three exons.

40. The vector system of Claim 22, wherein the unique region of DNA that provides for homologous recombination is a bacterial DNA, an insect DNA, a viral DNA or a synthetic DNA.

41. The vector system of Claim 40, wherein the unique region of DNA does not contain any functional genes.

DESMOND



HD = Salmonella HisD Gene
N3 = Neomycin Phosphotransferase Exon 3
D = Murine Dihydrofolate reductase
E = Cytomegalovirus and SV40 Enhancers
SA = Splice acceptor
BT = Mouse Beta Globin Major Promoter
B = Bovine Growth Hormone Polyadenylation
S = SV40 Early Polyadenylation
SV = SV40 Late Polyadenylation

FIGURE 1A

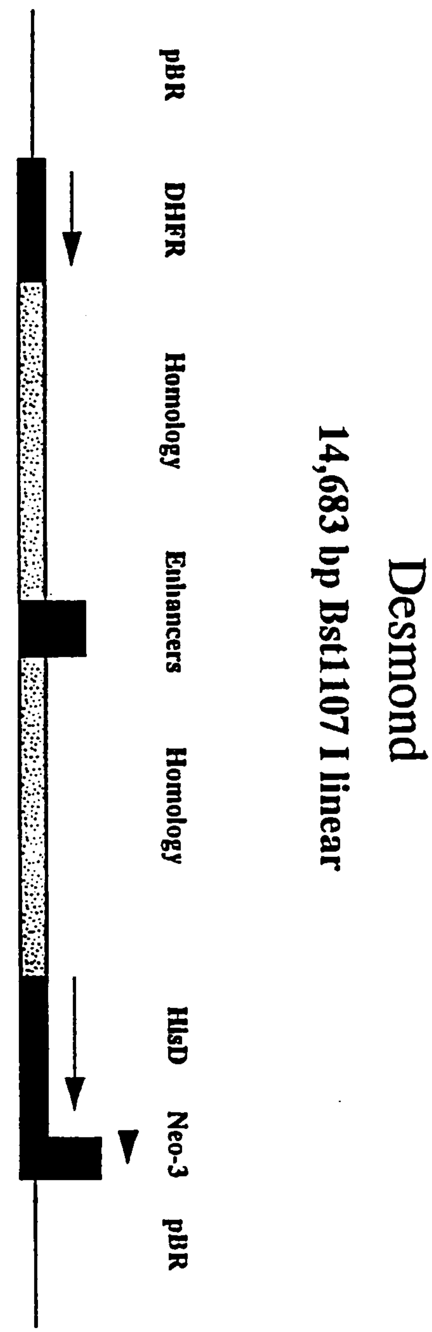
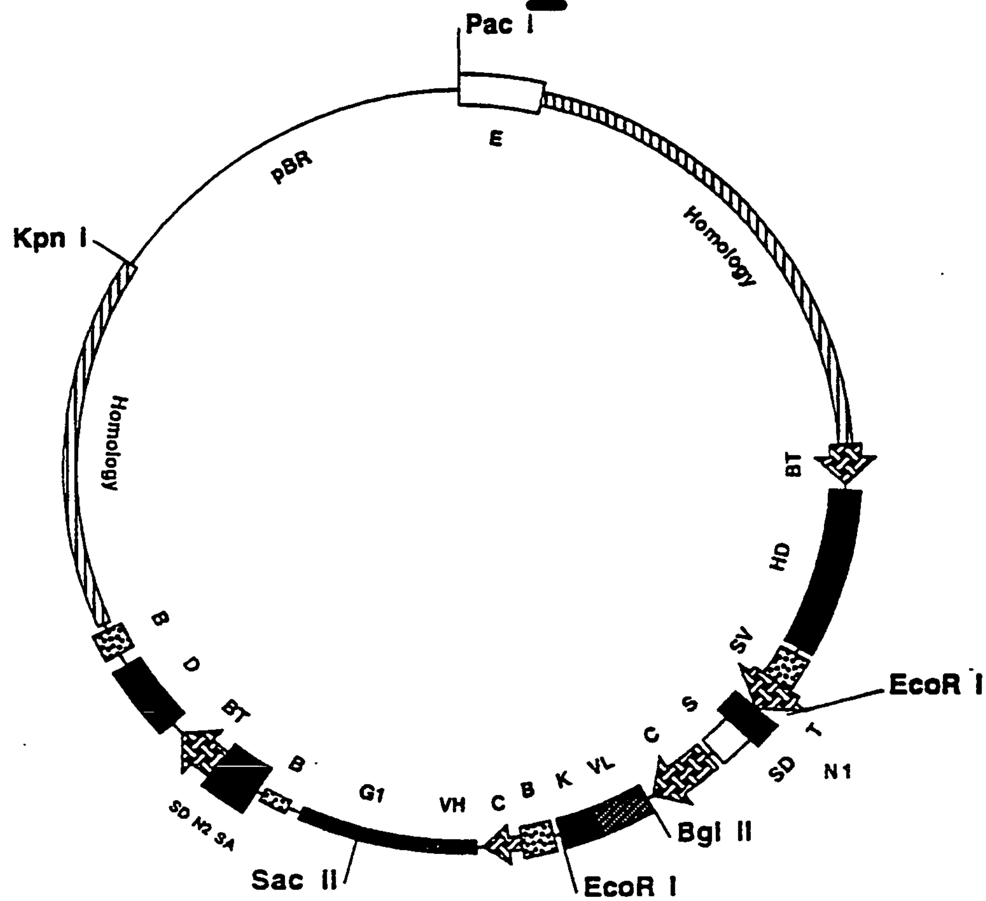

Desmond
14,683 bp Bst1107 I linear
pBR
DHFR
Homology
Enhancers
Homology
HisD
Neo-3
pBR

FIGURE 1B

Molly



D = Dihydrofolate reductase
N1 = Neomycin Phosphotransferase Exon 1
N2 = Neomycin Phosphotransferase Exon 2
VL = Anti-CD20 Light chain leader + Variable
K = Human Kappa Constant
VH = Anti-CD20 Heavy chain Leader + Variable
G1 = Human Gamma 1 Constant
HD = Salmonella Histidinol Dehydrogenase
E = CMV and SV40 enhancers
S = SV40 Origin
SD = Splice donor
SA = Splice acceptor
C = CMV promoter/enhancer
T = HSV TK promoter and Polyoma enhancers
BT = Mouse Beta Globin Major Promoter
SV = SV40 Late Polyadenylation
B = Bovine Growth Hormone Polyadenylation

FIGURE 2A

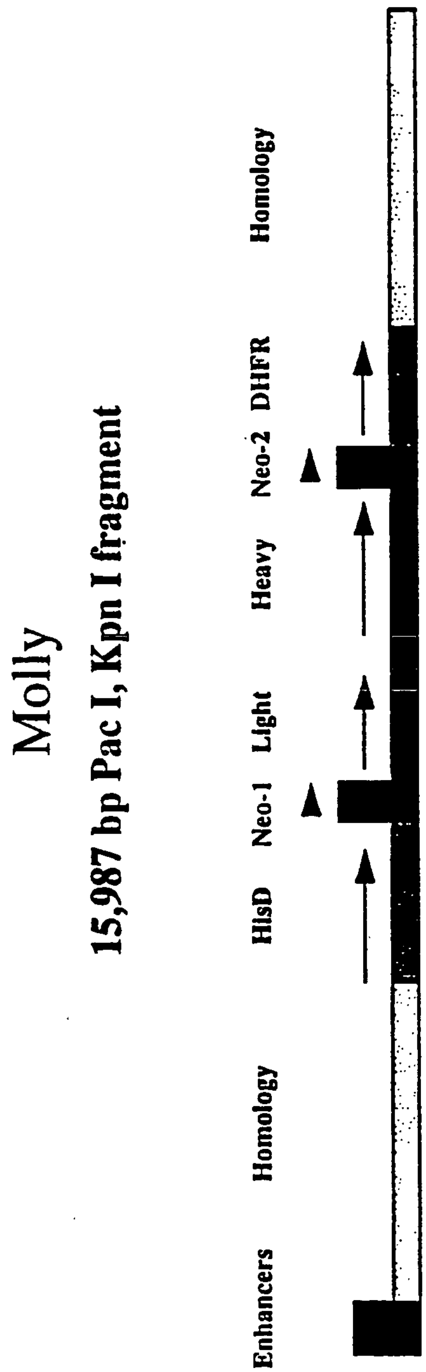

Molly
15,987 bp Pac I, Kpn I fragment
Enhancers
Homology
HisD
Neo-1
Light
Heavy
Neo-2
DHFR
Homology

FIGURE 2B

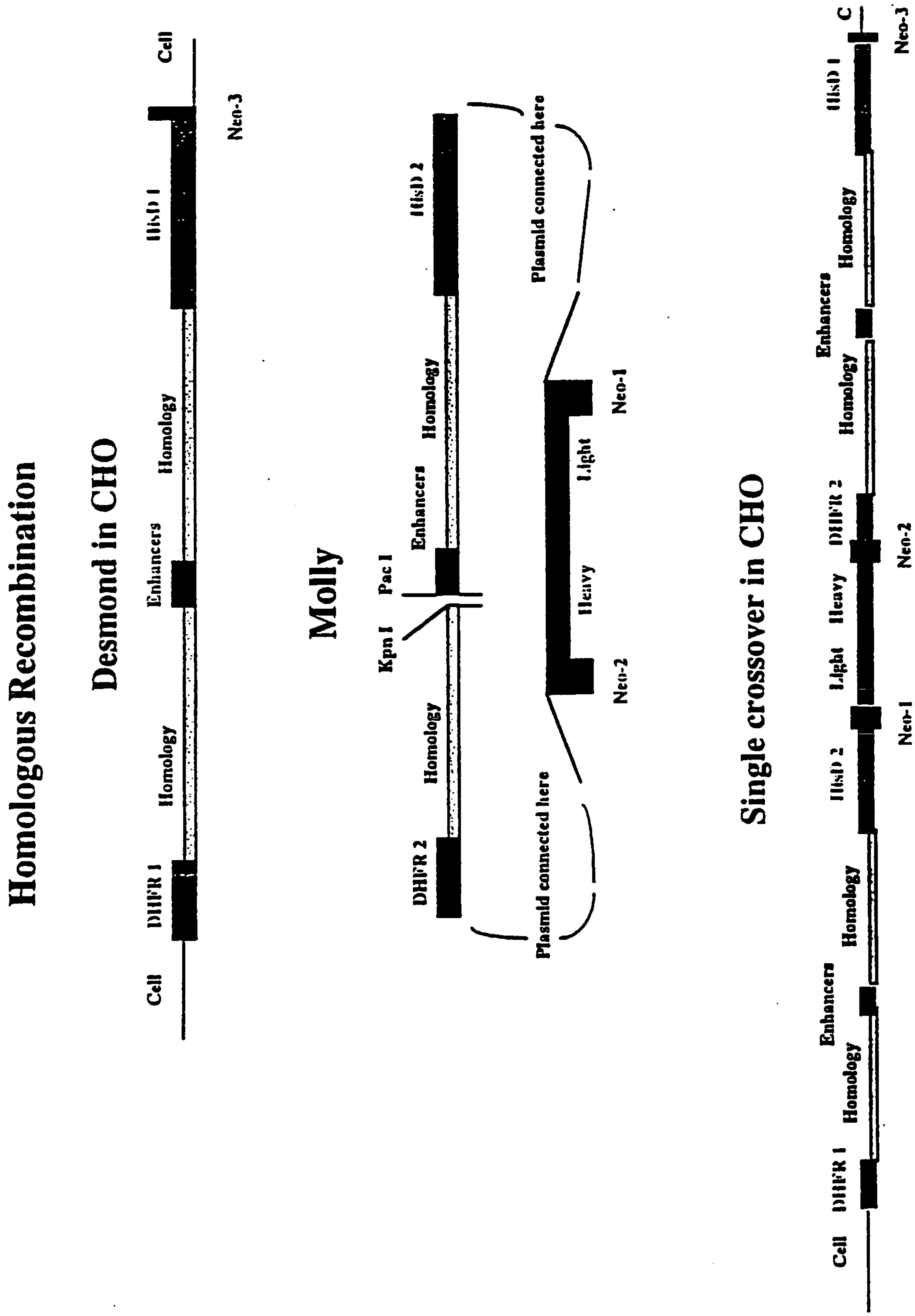

Homologous Recombination
Desmond in CHO
Cell
DHFR 1
Homology
Enhancers
Homology
HisD 1
Neo-3
Cell
Molly
DHFR 2
Homology
Kpn I
Pac I
Enhancers
Homology
HisD 2
Plasmid connected here
Neo-2
Heavy
Light
Neo-1
Plasmid connected here
Single crossover in CHO
Cell
DHFR 1
Homology
Enhancers
Homology
HisD 2
Neo-1
Light
Heavy
Neo-2
DHFR 2
Homology
Enhancers
Homology
HisD 1
C
Neo-3

FIGURE 7

Southern Analvsis of Desmond Marked CHO Cells

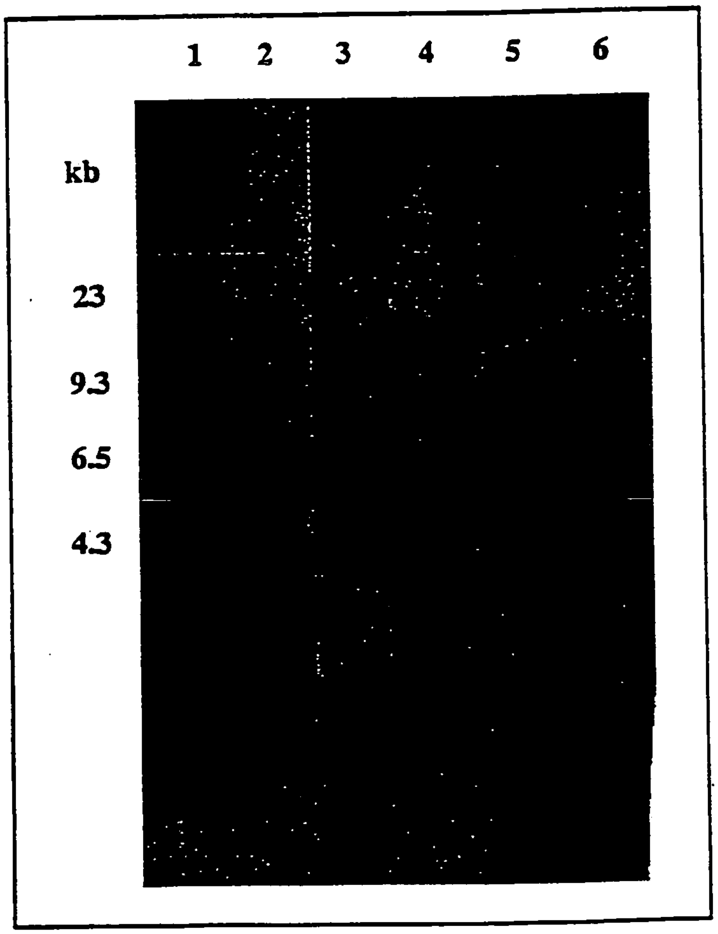


FIGURE 4

Northern Analysis of Desmond Marked CHO Cells

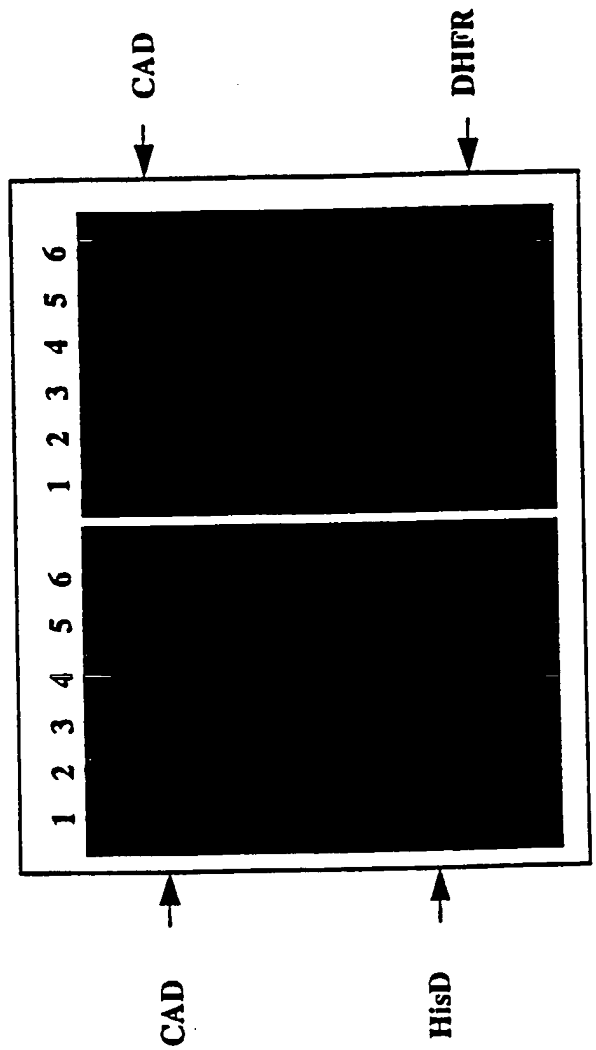


FIGURE 5

Southern Analysis of Anti CD20 Integrants in Marked CHO Cells

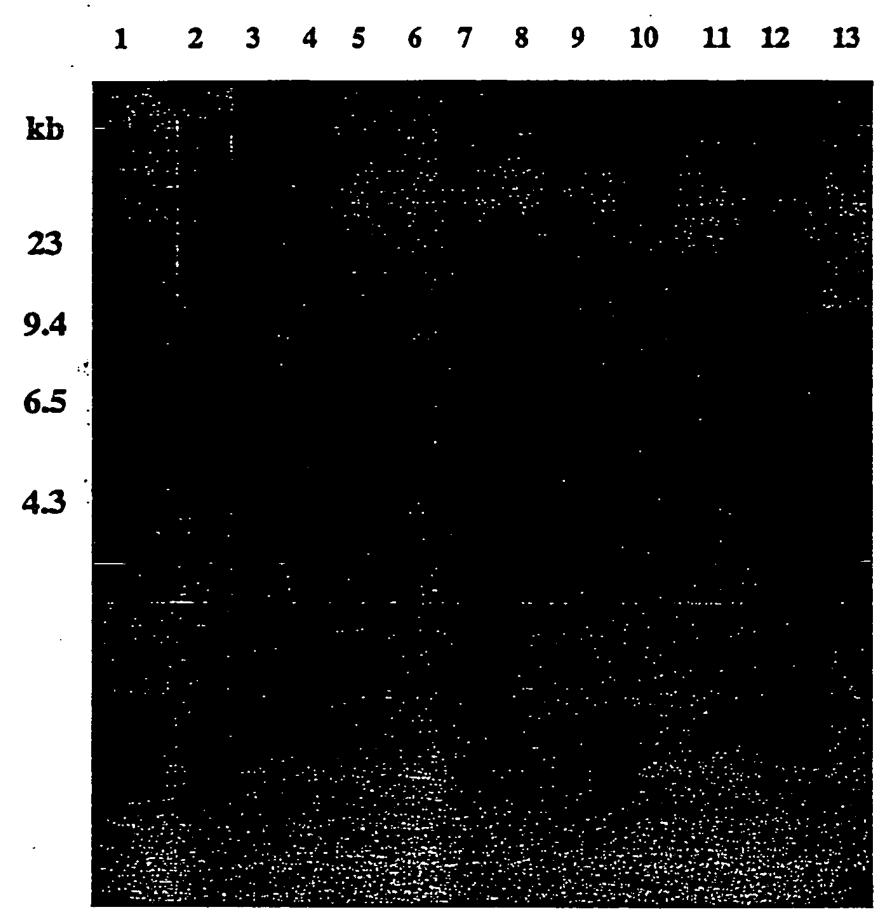


FIGURE 6

DNASIS
Desmond Lark

FIGURE 7

```
        10         20         30         40         50         60
TTTCTAGACC TAGGGCGGCC AGCTAGTAGC TTTGCTTCTC AATTTCTTAT TTGCATAATG

        70         80         90        100        110        120
AGAAAAAAAG GAAAATTAAT TTTAACACCA ATTCAGTAGT TGATTGAGCA AATGCGTTGC

       130        140        150        160        170        180
CAAAAAGGAT GCTTTAGAGA CAGTGTTCTC TGCACAGATA AGGACAAACA TTATTCAGAG

       190        200        210        220        230        240
GGAGTACCCA GAGCTGAGAC TCCTAAGCCA GTGAGTGGCA CAGCATCCAG GGAGAAATAT

       250        260        270        280        290        300
GCTTGTCATC ACCGAAGCCT GATTCCGTAG AGCCACACCC TGGTAAGGGC CAATCTGCTC

       310        320        330        340        350        360
ACACAGGATA GAGAGGGCAG GAGCCAGGGC AGAGCATATA AGGTGAGGTA GGATCAGTTG

       370        380        390        400        410        420
   CTCACAT TTGCTTCTGA CATAGTTGTG TTGGGAGCTT GGATAGCTTG GGGGGGGGAC

       430        440        450        460        470        480
AGCTCAGGGC TGCGATTTCG CGCCAAACTT GACGGCAATC CTAGCGTGAA GGCTGGTAGG

       490        500        510        520        530        540
ATTTTATCCC CGCTGCCATC ATGGTTCGAC CATTGAACTG CATCGTCGCC GTGTCCCAAA

       550        560        570        580        590        600
ATATGGGGAT TGGCAAGAAC GGAGACCTAC CCTGGCCTCC GCTCAGGAAC GAGTTCAAGT

       610        620        630        640        650        660
ACTTCCAAAG AATGACCACA ACCTCTTCAG TGGAAGGTAA ACAGAATCTG GTGATTATGG

       670        680        690        700        710        720
GTAGGAAAAC CTGGTTCTCC ATTCCTGAGA AGAATCGACC TTTAAAGGAC AGAATTAATA

       730        740        750        760        770        780
  ;TTCTCAG TAGAGAACTC AAAGAACCAC CACGAGGAGC TCATTTTCTT GCCAAAAGTT

       790        800        810        820        830        840
TGGATGATGC CTTAAGACTT ATTGAACAAC CGGAATTGGC AAGTAAAGTA GACATGGTTT

       850        860        870        880        890        900
GGATAGTCGG AGGCAGTTCT GTTTACCAGG AAGCCATGAA TCAACCAGGC CACCTCAGAC

       910        920        930        940        950        960
TCTTTGTGAC AAGGATCATG CAGGAATTTG AAAGTGACAC GTTTTTCCCA GAAATTGATT

       970        980        990       1000       1010       1020
TGGGGAAATA TAAACTTCTC CCAGAATACC CAGGCGTCCT CTCTGAGGTC CAGGAGGAAA

      1030       1040       1050       1060       1070       1080
AAGGCATCAA GTATAAGTTT GAAGTCTACG AGAAGAAAGA CTAACAGGAA GATGCTTTCA

      1090       1100       1110       1120       1130       1140
AGTTCTCTGC TCCCCTCCTA AAGCTATGCA TTTTTATAAG ACCATGGGAC TTTTGCTGGC

      1150       1160       1170       1180       1190       1200
TTTAGATCAG CCTCGACTGT GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC

      1210       1220       1230       1240       1250       1260
GTGCCTTCCT TGACCCTGGA AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA

      1270       1280       1290       1300       1310       1320
ATTGCATCGC ATTGTCTGAG TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC
```

DNASIS
Desmond Lark

1330 1340 1350 1360 1370 1380
AGCAAGGGGG AGGATTGGGA AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG

1390 1400 1410 1420 1430 1440
GCTTCTGAGG CGGAAAGAAC CAGCTGGGGC TCGAAGCGGC CGCCCATTTC GCTGGTGGTC

1450 1460 1470 1480 1490 1500
AGATGCGGGA TGGCGTGGGA CGCGGCGGGG AGCGTCACAC TGAGGTTTTC CGCCAGACGC

1510 1520 1530 1540 1550 1560
CACTGCTGCC AGGCGCTGAT GTGCCCGGCT TCTGACCATG CGGTCGCGTT CGGTTGCACT

1570 1580 1590 1600 1610 1620
ACGCGTACTG TGAGCCAGAG TTGCCCGGCG CTCTCCGGCT GCGGTAGTTC AGGCAGTTCA

1630 1640 1650 1660 1670 1680
ATCAACTGTT TACCTTGTGG AGCGACATCC AGAGGCACTT CACCGCTTGC CAGCGGCTTA

1690 1700 1710 1720 1730 1740
ATCCAGCG CCACCATCCA GTGCAGGAGC TCGTTATCGC TATGACGGAA CAGGTATTCG

1750 1760 1770 1780 1790 1800
CTGGTCACTT CGATGGTTTG CCCGGATAAA CGGAACTGGA AAAACTGCTG CTGGTGTTTT

1810 1820 1830 1840 1850 1860
GCTTCCGTCA GCGCTGGATG CGGCGTGCGG TCGGCAAAGA CCAGACCGTT CATACAGAAC

1870 1880 1890 1900 1910 1920
TGGCGATCGT TCGGCGTATC GCCAAAATCA CCGCCGTAAG CCGACCACGG GTTGCCGTTT

1930 1940 1950 1960 1970 1980
TCATCATATT TAATCAGCGA CTGATCCACC CAGTCCCAGA CGAAGCCGCC CTGTAAACGG

1990 2000 2010 2020 2030 2040
GGATACTGAC GAAACGCCTG CCAGTATTTA GCGAAACCGC CAAGACTGTT ACCCATCGCG

2050 2060 2070 2080 2090 2100
GGCGTATT CGCAAAGGAT CAGCGGGCGC GTCTCTCCAG GTAGCGAAAG CCATTTTTTG

2110 2120 2130 2140 2150 2160
ATGGACCATT TCGGCACAGC CGGGAAGGGC TGGTCTTCAT CCACGCGCGC GTACATCGGG

2170 2180 2190 2200 2210 2220
CAAATAATAT CGGTGGCCGT GGTGTCGGCT CCGCCGCCTT CATACTGCAC CGGGCGGGAA

2230 2240 2250 2260 2270 2280
GGATCGACAG ATTTGATCCA GCGATACAGC GCGTCGTGAT TAGCGCCGTG GCCTGATTCA

2290 2300 2310 2320 2330 2340
TTCCCCAGCG ACCAGATGAT CACACTCGGG TGATTACGAT CGCGCTGCAC CATTCGCGTT

2350 2360 2370 2380 2390 2400
ACGCGTTCGC TCATCGCCGG TAGCCAGCGC GGATCATCGG TCAGACGATT CATTGGCACC

2410 2420 2430 2440 2450 2460
ATGCCGTGGG TTTCAATATT GGCTTCATCC ACCACATACA GGCCGTAGCG GTCGCACAGC

2470 2480 2490 2500 2510 2520
GTGTACCACA GCGGATGGTT CGGATAATGC GAACAGCGCA CGGCGTTAAA GTTGTTCTGC

2530 2540 2550 2560 2570 2580
TTCATCAGCA GGATATCCTG CACCATCGTC TGCTCATCCA TGACCTGACC ATGCAGAGGA

2590 2600 2610 2620 2630 2640

DNASIS
Desmond Lark

```
TGATGCTCGT GACGGTTAAC GCCTCGAATC AGCAACGGCT TGCCGTTCAG CAGCAGCAGA

      2650       2660       2670       2680       2690       2700
CCATTTTCAA TCCGCACCTC GCGGAAACCG ACATCGCAGG CTTCTGCTTC AATCAGCGTG

      2710       2720       2730       2740       2750       2760
CCGTCGGCGG TGTGCAGTTC AACCACCGCA CGATAGAGAT TCGGGATTTC GGCGCTCCAC

      2770       2780       2790       2800       2810       2820
AGTTTCGGGT TTTCGACGTT CAGACGTAGT GTGACGCGAT CGGCATAACC ACCACGCTCA

      2830       2840       2850       2860       2870       2880
TCGATAATTT CACCGCCGAA AGGCGCGGTG CCGCTGGCGA CCTGCGTTTC ACCCTGCCAT

      2890       2900       2910       2920       2930       2940
AAAGAAACTG TTACCCGTAG GTAGTCACGC AACTCGCCGC ACATCTGAAC TTCAGCCTCC

      2950       2960       2970       2980       2990       3000
AGTACAGCGC GGCTGAAATC ATCATTAAAG CGAGTGGCAA CATGGAAATC GCTGATTTGT

      3010       3020       3030       3040       3050       3060
GTAGTCGGTT TATGCAGCAA CGAGACGTCA CGGAAAATGC CGCTCATCCG CCACATATCC

      3070       3080       3090       3100       3110       3120
TGATCTTCCA GATAACTGCC GTCACTCCAG CGCAGCACCA TCACCGCGAG GCGGTTTTCT

      3130       3140       3150       3160       3170       3180
CCGGCGCGTA AAAATGCGCT CAGGTCAAAT TCAGACGGCA AACGACTGTC CTGGCCGTAA

      3190       3200       3210       3220       3230       3240
CCGACCCAGC GCCCGTTGCA CCACAGATGA AACGCCGAGT TAACGCCATC AAAAATAATT

      3250       3260       3270       3280       3290       3300
CGCGTCTGGC CTTCCTGTAG CCAGCTTTCA TCAACATTAA ATGTGAGCGA GTAACAACCC

      3310       3320       3330       3340       3350       3360
GTCGGATTCT CCGTGGGAAC AAACGGCGGA TTGACCGTAA TGGGATAGGT CACGTTGGTG

      3370       3380       3390       3400       3410       3420
IAGATGGGCG CATCGTAACC GTGCATCTGC CAGTTTGAGG GGACGACGAC AGTATCGGCC

      3430       3440       3450       3460       3470       3480
TCAGGAAGAT CGCACTCCAG CCAGCTTTCC GGCACCGCTT CTGGTGCCGG AAACCAGGCA

      3490       3500       3510       3520       3530       3540
AAGCGCCATT CGCCATTCAG GCTGCGCAAC TGTTGGGAAG GGCGATCGGT GCGGGCCTCT

      3550       3560       3570       3580       3590       3600
TCGCTATTAC GCCAGCTGGC GAAAGGGGGA TGTGCTGCAA GGCGATTAAG TTGGGTAACG

      3610       3620       3630       3640       3650       3660
CCAGGGTTTT CCCAGTCACG ACGTTGTAAA ACGACTTAAT CCGTCGAGGG GCTGCCTCGA

      3670       3680       3690       3700       3710       3720
AGCAAACGAC CTTCCGTTGT GCAGCCAGCG GCGCCTGCGC CGGTGCCCAC AATCGTGCGC

      3730       3740       3750       3760       3770       3780
GAACAAACTA AACCAGAACA AATTATACCG GCGGCACCGC CGCCACCACC TTCTCCCGTG

      3790       3800       3810       3820       3830       3840
CCTAACATTC CAGCGCCTCC ACCACCACCA CCACCATCGA TGTCTGAATT GCCGCCCGCT

      3850       3860       3870       3880       3890       3900
CCACCAATGC CGACGGAACC TCAACCCGCT GCACCTTTAG ACGACAGACA ACAATTGTTG
```

DNASIS
Desmond Lark

3910 3920 3930 3940 3950 3960
GAAGCTATTA GAAACGAAAA AAATCGCACT CGTCTCAGAC CGGCTCTCTT AAGGTAGCTC

3970 3980 3990 4000 4010 4020
AAACCAAAAA CGGCGCCCGA AACCAGTACA ATAGTTGAGG TGCCGACTGT GTTGCCTAAA

4030 4040 4050 4060 4070 4080
GAGACATTTG AGCCTAAACC GCCGTCTGCA TCACCGCCAC CACCTCCGCC TCCGCCTCCG

4090 4100 4110 4120 4130 4140
CCGCCAGCCC CGCCTGCGCC TCCACCGATG GTAGATTTAT CATCAGCTCC ACCACCGCCG

4150 4160 4170 4180 4190 4200
CCATTAGTAG ATTTGCCGTC TGAAATGTTA CCACCGCCTG CACCATCGCT TTCTAACGTG

4210 4220 4230 4240 4250 4260
TTGTCTGAAT TAAAATCGGG CACAGTTAGA TTGAAACCCG CCCAAAAACG CCCGCAATCA

4270 4280 4290 4300 4310 4320
ATAATTC CAAAAAGCTC AACTACAAAT TTGATCGCGG ACGTGTTAGC CGACACAATT

4330 4340 4350 4360 4370 4380
AATAGGCGTC GTGTGGCTAT GGCAAAATCG TCTTCGGAAG CAACTTCTAA CGACGAGGGT

4390 4400 4410 4420 4430 4440
TGGGACGACG ACGATAATCG GCCTAATAAA GCTAACACGC CCGATGTTAA ATATGTCCAA

4450 4460 4470 4480 4490 4500
GCTACTAGTG GTACCTTAAT TAAGGGGCGG AGAATGGGCG GAACTGGGCG GAGTTAGGGG

4510 4520 4530 4540 4550 4560
CGGGATGGGC GGAGTTAGGG GCGGGACTAT GGTTGCTGAC TAATTGAGAT GCATGCTTTG

4570 4580 4590 4600 4610 4620
CATACTTCTG CCTGCTGGGG AGCCTGGGGA CTTTCCACAC CTGGTTGCTG ACTAATTGAG

4630 4640 4650 4660 4670 4680
GCATGCTT TGCATACTTC TGCCTGCTGG GGAGCCTGGG GACTTTCCAC ACCCTAACTG

4690 4700 4710 4720 4730 4740
ACACACATTC CACAGAATTA ATTCCCCTAG TTATTAATAG TAATCAATTA CGGGGTCATT

4750 4760 4770 4780 4790 4800
AGTTCATAGC CCATATATGG AGTTCCGCGT TACATAACTT ACGGTAAATG GCCCGCCTGG

4810 4820 4830 4840 4850 4860
CTGACCGCCC AACGACCCCC GCCCATTGAC GTCAATAATG ACGTATGTTC CCATAGTAAC

4870 4880 4890 4900 4910 4920
GCCAATAGGG ACTTTCCATT GACGTCAATG GGTGGAGTAT TTACGGTAAA CTGCCCACTT

4930 4940 4950 4960 4970 4980
GGCAGTACAT CAAGTGTATC ATATGCCAAG TACGCCCCCT ATTGACGTCA ATGACGGTAA

4990 5000 5010 5020 5030 5040
ATGGCCCGCC TGGCATTATG CCCAGTACAT GACCTTATGG GACTTTCCTA CTTGGCAGTA

5050 5060 5070 5080 5090 5100
CATCTACGTA TTAGTCATCG CTATTACCAT GGTGATGCGG TTTTGGCAGT ACATCAATGG

5110 5120 5130 5140 5150 5160
GCGTGGATAG CGGTTTGACT CACGGGGATT TCCAAGTCTC CACCCCATTG ACGTCAATGG

5170 5180 5190 5200 5210 5220
GAGTTTGTTT TGAAGCTTGG CCGGCCAGCT TTATTTAACG TGTTTACGTC GAGTCAATTG

DNASIS
Desmond Lark

```
      5230      5240      5250      5260      5270      5280
TACACTAACG ACAGTGATGA AAGAAATACA AAAGCGCATA ATATTTTGAA CGACGTCGAA

      5290      5300      5310      5320      5330      5340
CCTTTATTAC AAAACAAAAC ACAAACGAAT ATCGACAAAG CTAGATTGCT GCTACAAGAT

      5350      5360      5370      5380      5390      5400
TTGGCAAGTT TTGTGGCGTT GAGCGAAAAT CCATTAGATA GTCCAGCCAT CGGTTCGGAA

      5410      5420      5430      5440      5450      5460
AAACAACCCT TGTTTGAAAC TAATCGAAAC CTATTTTACA AATCTATTGA GGATTTAATA

      5470      5480      5490      5500      5510      5520
TTTAAATTCA GATATAAAGA CGCTGAAAAT CATTTGATTT TCGCTCTAAC ATACCACCCT

      5530      5540      5550      5560      5570      5580
AAAGATTATA AATTTAATGA ATTATTAAAA TACATCAGCA ACTATATATT GATAGACATT

      5590      5600      5610      5620      5630      5640
. .CAGTTTGT GATATTAGTT TGTGCGTCTC ATTACAATGG CTGTTATTTT TAACAACAAA

      5650      5660      5670      5680      5690      5700
CAACTGCTCG CAGACAATAG TATAGAAAAG GGAGGTGAAC TGTTTTTGTT TAACGGTTCG

      5710      5720      5730      5740      5750      5760
TACAACATTT TGGAAAGTTA TGTTAATCCG GTGCTGCTAA AAAATGGTGT AATTGAACTA

      5770      5780      5790      5800      5810      5820
GAAGAAGCTG CGTACTATGC CGGCAACATA TTGTACAAAA CCGACGATCC CAAATTCATT

      5830      5840      5850      5860      5870      5880
GATTATATAA ATTTAATAAT TAAAGCAACA CACTCCGAAG AACTACCAGA AAATAGCACT

      5890      5900      5910      5920      5930      5940
GTTGTAAATT ACAGAAAAAC TATGCGCAGC GGTACTATAC ACCCCATTAA AAAAGACATA

      5950      5960      5970      5980      5990      6000
...TATTTATG ACAACAAAAA ATTTACTCTA TACGATAGAT ACATATATGG ATACGATAAT

      6010      6020      6030      6040      6050      6060
AACTATGTTA ATTTTTATGA GGAGAAAAAT GAAAAAGAGA AGGAATACGA AGAAGAAGAC

      6070      6080      6090      6100      6110      6120
GACAAGGCGT CTAGTTTATG TGAAAATAAA ATTATATTGT CGCAAATTAA CTGTGAATCA

      6130      6140      6150      6160      6170      6180
TTTGAAAATG ATTTTAAATA TTACCTCAGC GATTATAACT ACGCGTTTTC AATTATAGAT

      6190      6200      6210      6220      6230      6240
AATACTACAA ATGTTCTTGT TGCGTTTGGT TTGTATCGTT AATAAAAAAC AAATTTGACA

      6250      6260      6270      6280      6290      6300
TTTATAATTG TTTTATTATT CAATAATTAC AAATAGGATT GAGACCCTTG CAGTTGCCAG

      6310      6320      6330      6340      6350      6360
CAAACGGACA GAGCTTGTCG AGGAGAGTTG TTGATTCATT GTTTGCCTCC CTGCTGCGGT

      6370      6380      6390      6400      6410      6420
TTTTCACCGA AGTTCATGCC AGTCCAGCGT TTTTGCAGCA GAAAAGCCGC CGACTTCGGT

      6430      6440      6450      6460      6470      6480
TTGCGGTCGC GAGTGAAGAT CCCTTTCTTG TTACCGCCAA CGCGCAATAT GCCTTGCGAG

      6490      6500      6510      6520      6530      6540
```

DNASIS
Desmond Lark

GTCGCAAAAT CGGCGAAATT CCATACCTGT TCACCGACGA CGGCGCTGAC GCGATCAAAG

6550 6560 6570 6580 6590 6600
ACGCGGTGAT ACATATCCAG CCATGCACAC TGATACTCTT CACTCCACAT GTCGGTGTAC

6610 6620 6630 6640 6650 6660
ATTGAGTGCA GCCCGGCTAA CGTATCCACG CCGTATTCGG TGATGATAAT CGGCTGATGC

6670 6680 6690 6700 6710 6720
AGTTTCTCCT GCCAGGCCAG AAGTTCTTTT TCCAGTACCT TCTCTGCCGT TTCCAAATCG

6730 6740 6750 6760 6770 6780
CCGCTTTGGA CATACCATCC GTAATAACGG TTCAGGCACA GCACATCAAA GAGATCGCTG

6790 6800 6810 6820 6830 6840
ATGGTATCGG TGTGAGCGTC GCAGAACATT ACATTGACGC AGGTGATCGG ACGCGTCGGG

6850 6860 6870 6880 6890 6900
TCGAGTTTAC GCGTTGCTTC CGCCAGTGGC GCGAAATATT CCCGTGCACC TTGCGGACGG

6910 6920 6930 6940 6950 6960
GTATCCGGTT CGTTGGCAAT ACTCCACATC ACCACGCTTG GGTGGTTTTT GTCACGCGCT

6970 6980 6990 7000 7010 7020
ATCAGCTCTT TAATCGCCTG TAAGTGCGCT TGCTGAGTTT CCCCGTTGAC TGCCTCTTCG

7030 7040 7050 7060 7070 7080
CTGTACAGTT CTTTCGGCTT GTTGCCCGCT TCGAAACCAA TGCCTAAAGA GAGGTTAAAG

7090 7100 7110 7120 7130 7140
CCGACAGCAG CAGTTTCATC AATCACCACG ATGCCATGTT CATCTGCCCA GTCGAGCATC

7150 7160 7170 7180 7190 7200
TCTTCAGCGT AAGGGTAATG CGAGGTACGG TAGGAGTTGG CCCCAATCCA GTCCATTAAT

7210 7220 7230 7240 7250 7260
GCGTGGTCGT GCACCATCAG CACGTTATCG AATCCTTTGC CACGCAAGTC CGCATCTTCA

7270 7280 7290 7300 7310 7320
TGACGACCAA AGCCAGTAAA GTAGAACGGT TTGTGGTTAA TCAGGAACTG TTCGCCCTTC

7330 7340 7350 7360 7370 7380
ACTGCCACTG ACCGGATGCC GACGCGAAGC GGGTAGATAT CACACTCTGT CTGGCTTTTG

7390 7400 7410 7420 7430 7440
GCTGTGACGC ACAGTTCATA GAGATAACCT TCACCCGGTT GCCAGAGGTG CGGATTCACC

7450 7460 7470 7480 7490 7500
ACTTGCAAAG TCCCGCTAGT GCCTTGTCCA GTTGCAACCA CCTGTTGATC CGCATCACGC

7510 7520 7530 7540 7550 7560
AGTTCAACGC TGACATCACC ATTGGCCACC ACCTGCCAGT CAACAGACGC GTGGTTACAG

7570 7580 7590 7600 7610 7620
TCTTGCGCGA CATGCGTCAC CACGGTGATA TCGTCCACCC AGGTGTTCGG CGTGGTGTAG

7630 7640 7650 7660 7670 7680
AGCATTACGC TGCGATGGAT TCCGGCATAG TTAAAGAAAT CATGGAAGTA AGACTGCTTT

7690 7700 7710 7720 7730 7740
TTCTTGCCGT TTTCGTCGGT AATCACCATT CCCGGCGGGA TAGTCTGCCA GTTCAGTTCG

7750 7760 7770 7780 7790 7800
TTGTTCACAC AAACGGTGAT ACCCCTCGAC GGATTAAAGA CTTCAAGCGG TCAACTATGA

DNASIS
Desmond Lark

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      7810       7820       7830       7840       7850       7860
AGAAGTGTTC GTCTTCGTCC CAGTAAGCTA TGTCTCCAGA ATGTAGCCAT CCATCCTTGT

      7870       7880       7890       7900       7910       7920
CAATCAAGGC GTTGGTCGCT TCCGGATTGT TTACATAACC GGACATAATC ATAGGTCCTC

      7930       7940       7950       7960       7970       7980
TGACACATAA TTCGCCTCTC TGATTAACGC CCAGCGTTTT CCCGGTATCC AGATCCACAA

      7990       8000       8010       8020       8030       8040
CCTTCGCTTC AAAAAATGGA ACAACTTTAC CGACCGCGCC CGGTTTATCA TCCCCCTCGG

      8050       8060       8070       8080       8090       8100
GTGTAATCAG AATAGCTGAT GTAGTCTCAG TGAGCCCATA TCCTTGTCGT ATCCCTGGAA

      8110       8120       8130       8140       8150       8160
GATGGAAGCG TTTTGCAACC GCTTCCCCGA CTTCTTTCGA AAGAGGTGCG CCCCCAGAAG

      8170       8180       8190       8200       8210       8220
  ATTTCGTG TAAATTAGAT AAATCGTATT TGTCAATCAG AGTGCTTTTG GCGAAGAATG

      8230       8240       8250       8260       8270       8280
AAAATAGGGT TGGTACTAGC AACGCACTTT GAATTTTGTA ATCCTGAAGG GATCGTAAAA

      8290       8300       8310       8320       8330       8340
ACAGCTCTTC TTCAAATCTA TACATTAAGA CGACTCGAAA TCCACATATC AAATATCCGA

      8350       8360       8370       8380       8390       8400
GTGTAGTAAA CATTCCAAAA CCGTGATGGA ATGGAACAAC ACTTAAAATC GCAGTATCCG

      8410       8420       8430       8440       8450       8460
GAATGATTTG ATTGCCAAAA ATAGGATCTC TGGCATGCGA GAATCTGACG CAGGCAGTTC

      8470       8480       8490       8500       8510       8520
TATGCGGAAG GGCCACACCC TTAGGTAACC CAGTAGATCC AGAGGAATTG TTTTGTCACG

      8530       8540       8550       8560       8570       8580
  CAAAGGAC TCTGGTACAA AATCGTATTC ATTAAAACCG GGAGGTAGAT GAGATGTGAC

      8590       8600       8610       8620       8630       8640
GAACGTGTAC ATCGACTGAA ATCCCTGGTA ATCCGTTTTA GAATCCATGA TAATAATTTT

      8650       8660       8670       8680       8690       8700
CTGGATTATT GGTAATTTTT TTTGCACGTT CAAAATTTTT TGCAACCCCT TTTTGGAAAC

      8710       8720       8730       8740       8750       8760
AAACACTACG GTAGGCTGCG AAATGTTCAT ACTGTTGAGC AATTCACGTT CATTATAAAT

      8770       8780       8790       8800       8810       8820
GTCGTTCGCG GGCGCAACTG CAACTCCGAT AAATAACGCG CCCAACACCG GCATAAAGAA

      8830       8840       8850       8860       8870       8880
TTGAAGAGAG TTTTCACTGC ATACGACGAT TCTGTGATTT GTATTCAGCC CATATCGTTT

      8890       8900       8910       8920       8930       8940
CATAGCTTCT GCCAACCGAA CGGACATTTC GAAGTATTCC GCGTACGTGA TGTTCACCTC

      8950       8960       8970       8980       8990       9000
GATATGTGCA TCTGTAAAAG GAATTGTTCC AGGAACCAGG GCGTATCTCT TCATAGCCTT

      9010       9020       9030       9040       9050       9060
ATGCAGTTGC TCTCCAGCGG TTCCATCCTC TAGCTTTGCT TCTCAATTTC TTATTTGCAT

      9070       9080       9090       9100       9110       9120
AATGAGAAAA AAAGGAAAAT TAATTTTAAC ACCAATTCAG TAGTTGATTG AGCAAATGCG
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DNASIS
Desmond Lark

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      9130       9140       9150       9160       9170       9180
TTGCCAAAAA GGATGCTTTA GAGACAGTGT TCTCTGCACA GATAAGGACA AACATTATTC

      9190       9200       9210       9220       9230       9240
AGAGGGAGTA CCCAGAGCTG AGACTCCTAA GCCAGTGAGT GGCACAGCAT CCAGGGAGAA

      9250       9260       9270       9280       9290       9300
ATATGCTTGT CATCACCGAA GCCTGATTCC GTAGAGCCAC ACCCTGGTAA GGGCCAATCT

      9310       9320       9330       9340       9350       9360
GCTCACACAG GATAGAGAGG GCAGGAGCCA GGGCAGAGCA TATAAGGTGA GGTAGGATCA

      9370       9380       9390       9400       9410       9420
GTTGCTCCTC ACATTTGCTT CTGACATAGT TGTGTTGGGA GCTTGGATCG ATCCACCATG

      9430       9440       9450       9460       9470       9480
GGCTTCAATA CCCTGATTGA CTGGAACAGC TGTAGCCCTG AACAGCAGCG TGCGCTGCTG

      9490       9500       9510       9520       9530       9540
A[illegible]CGTCCGG CGATTTCCGC CTCTGACAGT ATTACCCGGA CGGTCAGCGA TATTTTGGAT

      9550       9560       9570       9580       9590       9600
AATGTAAAAA CGCGCGGTGA CGATGCCCTG CGTGAATACA GCGCTAAATT TGATAAAACA

      9610       9620       9630       9640       9650       9660
GAAGTGACAG CGCTACGCGT CACCCCTGAA GAGATCGCCG CCGCCGGCGC GCGTCTGAGC

      9670       9680       9690       9700       9710       9720
GACGAATTAA AACAGGCGAT GACCGCTGCC GTCAAAAATA TTGAAACGTT CCATTCCGCG

      9730       9740       9750       9760       9770       9780
CAGACGCTAC CGCCTGTAGA TGTGGAAACC CAGCCAGGCG TGCGTTGCCA GCAGGTTACG

      9790       9800       9810       9820       9830       9840
CGTCCCGTCT CGTCTGTCGG TCTGTATATT CCCGGCGGCT CGGCTCCGCT CTTCTCAACG

      9850       9860       9870       9880       9890       9900
[illegible]CTGATGC TGGCGACGCC GGCGCGCATT GCGGGATGCC AGAAGGTGGT TCTGTGCTCG

      9910       9920       9930       9940       9950       9960
CCGCCGCCCA TCGCTGATGA AATCCTCTAT GCGGCGCAAC TGTGTGGCGT GCAGGAAATC

      9970       9980       9990      10000      10010      10020
TTTAACGTCG GCGGCGCGCA GGCGATTGCC GCTCTGGCCT TCGGCAGCGA GTCCGTACCG

     10030      10040      10050      10060      10070      10080
AAAGTGGATA AAATTTTTGG CCCCGGCAAC GCCTTTGTAA CCGAAGCCAA ACGTCAGGTC

     10090      10100      10110      10120      10130      10140
AGCCAGCGTC TCGACGGCGC GGCTATCGAT ATGCCAGCCG GGCCGTCTGA AGTACTGGTG

     10150      10160      10170      10180      10190      10200
ATCGCAGACA GCGGCGCAAC ACCGGATTTC GTCGCTTCTG ACCTGCTCTC CCAGGCTGAG

     10210      10220      10230      10240      10250      10260
CACGGCCCGG ATTCCCAGGT GATCCTGCTG ACGCCTGATG CTGACATTGC CCGCAAGGTG

     10270      10280      10290      10300      10310      10320
GCGGAGGCGG TAGAACGTCA ACTGGCGGAA CTGCCGCGCG CGGACACCGC CCGGCAGGCC

     10330      10340      10350      10360      10370      10380
CTGAGCGCCA GTCGTCTGAT TGTGACCAAA GATTTAGCGC AGTGCGTCGC CATCTCTAAT

     10390      10400      10410      10420      10430      10440
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DNASIS
Desmond Lark

CAGTATGGGC CGGAACACTT AATCATCCAG ACGCGCAATG CGCGCGATTT GGTGGATGCG

10450 10460 10470 10480 10490 10500
ATTACCAGCG CAGGCTCGGT ATTTCTCGGC GACTGGTCGC CGGAATCCGC CGGTGATTAC

10510 10520 10530 10540 10550 10560
GCTTCCGGAA CCAACCATGT TTTACCGACC TATGGCTATA CTGCTACCTG TTCCAGCCTT

10570 10580 10590 10600 10610 10620
GGGTTAGCGG ATTTCCAGAA ACGGATGACC GTTCAGGAAC TGTCGAAAGC GGGCTTTTCC

10630 10640 10650 10660 10670 10680
GCTCTGGCAT CAACCATTGA AACATTGGCG GCGGCAGAAC GTCTGACCGC CCATAAAAAT

10690 10700 10710 10720 10730 10740
GCCGTGACCC TGCGCGTAAA CGCCCTCAAG GAGCAAGCAT GAGCACTGAA AACACTCTCA

10750 10760 10770 10780 10790 10800
GCGTCGCTGA CTTAGCCCGT GAAAATGTCC GCAACCTGGA GATCCAGACA TGGATAAGAT

10810 10820 10830 10840 10850 10860
ACATTGATGA GTTTGGACAA ACCACAACTA GAATGCAGTG AAAAAAATGC TTTATTTGTG

10870 10880 10890 10900 10910 10920
AAATTTGTGA TGCTATTGCT TTATTTGTAA CCATTATAAG CTGCAATAAA CAAGTTAACA

10930 10940 10950 10960 10970 10980
ACAACAATTG CATTCATTTT ATGTTTCAGG TTCAGGGGGA GGTGTGGGAG GTTTTTTAAA

10990 11000 11010 11020 11030 11040
GCAAGTAAAA CCTCTACAAA TGTGGTATGG CTGATTATGA TCTCTAGGGC CGGCCCTCGA

11050 11060 11070 11080 11090 11100
CGGCGCGCCT GGCCGCTACT AACTCTCTCC TCCCTCCTTT TTCCTGCAGG CTCAAGGCGC

11110 11120 11130 11140 11150 11160
GCATGCCCGA CGGCGAGGAT CTCGTCGTGA CCCATGGCGA TGCCTGCTTG CCGAATATCA

11170 11180 11190 11200 11210 11220
TGGTGGAAAA TGGCCGCTTT TCTGGATTCA TCGACTGTGG CCGGCTGGGT GTGGCGGACC

11230 11240 11250 11260 11270 11280
GCTATCAGGA CATAGCGTTG GCTACCCGTG ATATTGCTGA AGAGCTTGGC GGCGAATGGG

11290 11300 11310 11320 11330 11340
CTGACCGCTT CCTCGTGCTT TACGGTATCG CCGCTCCCGA TTCGCAGCGC ATCGCCTTCT

11350 11360 11370 11380 11390 11400
ATCGCCTTCT TGACGAGTTC TTCTGAGCGG GACTCTGGGG TTCGAAATGA CCGACCAAGC

11410 11420 11430 11440 11450 11460
GACGCCCAAC CTGCCATCAC GAGATTTCGA TTCCACCGCC GCCTTCTATG AAAGGTTGGG

11470 11480 11490 11500 11510 11520
CTTCGGAATC GTTTTCCGGG ACGCCGGCTG GATGATCCTC CAGCGCGGGG ATCTCATGCT

11530 11540 11550 11560 11570 11580
GGAGTTCTTC GCCCACCCCA ACTTGTTTAT TGCAGCTTAT AATGGTTACA AATAAAGCAA

11590 11600 11610 11620 11630 11640
TAGCATCACA AATTTCACAA ATAAAGCATT TTTTTCACTG CATTCTAGTT GTGGTTTGTC

11650 11660 11670 11680 11690 11700
CAAACTCATC AATCTATCTT ATCATGTCTG GATCGCGGCC GGTCTCTCTC TAGCCCTAGG

DNASIS
Desmond Lark

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     11710      11720      11730      11740      11750      11760
TCTAGACTTG GCAGAACATA TCCATCGCGT CCGCCATCTC CAGCAGCCGC ACGCGGCGCA

     11770      11780      11790      11800      11810      11820
TCTCGGGCAG CGTTGGGTCC TGGCCACGGG TGCGCATGAT CGTGCTCCTG TCGTTGAGGA

     11830      11840      11850      11860      11870      11880
CCCGGCTAGG CTGGCGGGGT TGCCTTACTG GTTAGCAGAA TGAATCACCG ATACGCGAGC

     11890      11900      11910      11920      11930      11940
GAACGTGAAG CGACTGCTGC TGCAAAACGT CTGCGACCTG AGCAACAACA TGAATGGTCT

     11950      11960      11970      11980      11990      12000
TCGGTTTCCG TGTTTCGTAA AGTCTGGAAA CGCGGAAGTC AGCGCCCTGC ACCATTATGT

     12010      12020      12030      12040      12050      12060
TCCGGATCTG CATCGCAGGA TGCTGCTGGC TACCCTGTGG AACACCTACA TCTGTATTAA

     12070      12080      12090      12100      12110      12120
CGAAGCGCTG GCATTGACCC TGAGTGATTT TTCTCTGGTC CCGCCGCATC CATACCGCCA

     12130      12140      12150      12160      12170      12180
GTTGTTTACC CTCACAACGT TCCAGTAACC GGGCATGTTC ATCATCAGTA ACCCGTATCG

     12190      12200      12210      12220      12230      12240
TGAGCATCCT CTCTCGTTTC ATCGGTATCA TTACCCCCAT GAACAGAAAT CCCCCTTACA

     12250      12260      12270      12280      12290      12300
CGGAGGCATC AGTGACCAAA CAGGAAAAAA CCGCCCTTAA CATGGCCCGC TTTATCAGAA

     12310      12320      12330      12340      12350      12360
GCCAGACATT AACGCTTCTG GAGAAACTCA ACGAGCTGGA CGCGGATGAA CAGGCAGACA

     12370      12380      12390      12400      12410      12420
TCTGTGAATC GCTTCACGAC CACGCTGATG AGCTTTACCG CAGCTGCCTC GCGCGTTTCG

     12430      12440      12450      12460      12470      12480
GTGATGACGG TGAAAACCTC TGACACATGC AGCTCCCGGA GACGGTCACA GCTTGTCTGT

     12490      12500      12510      12520      12530      12540
AAGCGGATGC CGGGAGCAGA CAAGCCCGTC AGGGCGCGTC AGCGGGTGTT GGCGGGTGTC

     12550      12560      12570      12580      12590      12600
GGGGCGCAGC CATGACCCAG TCACGTAGCG ATAGCGGAGT GTATACTGGC TTAACTATGC

     12610      12620      12630      12640      12650      12660
GGCATCAGAG CAGATTGTAC TGAGAGTGCA CCATATGCGG TGTGAAATAC CGCACAGATG

     12670      12680      12690      12700      12710      12720
CGTAAGGAGA AAATACCGCA TCAGGCGCTC TTCCGCTTCC TCGCTCACTG ACTCGCTGCG

     12730      12740      12750      12760      12770      12780
CTCGGTCGTT CGGCTGCGGC GAGCGGTATC AGCTCACTCA AAGGCGGTAA TACGGTTATC

     12790      12800      12810      12820      12830      12840
CACAGAATCA GGGGATAACG CAGGAAAGAA CATGTGAGCA AAAGGCCAGC AAAAGGCCAG

     12850      12860      12870      12880      12890      12900
GAACCGTAAA AAGGCCGCGT TGCTGGCGTT TTTCCATAGG CTCCGCCCCC CTGACGAGCA

     12910      12920      12930      12940      12950      12960
TCACAAAAAT CGACGCTCAA GTCAGAGGTG GCGAAACCCG ACAGGACTAT AAAGATACCA

     12970      12980      12990      13000      13010      13020
GGCGTTTCCC CCTGGAAGCT CCCTCGTGCG CTCTCCTGTT CCGACCCTGC CGCTTACCGG
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DNASIS
Desmond Lark

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     13030      13040      13050      13060      13070      13080
ATACCTGTCC GCCTTTCTCC CTTCGGGAAG CGTGGCGCTT TCTCATAGCT CACGCTGTAG

     13090      13100      13110      13120      13130      13140
GTATCTCAGT TCGGTGTAGG TCGTTCGCTC CAAGCTGGGC TGTGTGCACG AACCCCCCGT

     13150      13160      13170      13180      13190      13200
TCAGCCCGAC CGCTGCGCCT TATCCGGTAA CTATCGTCTT GAGTCCAACC CGGTAAGACA

     13210      13220      13230      13240      13250      13260
CGACTTATCG CCACTGGCAG CAGCCACTGG TAACAGGATT AGCAGAGCGA GGTATGTAGG

     13270      13280      13290      13300      13310      13320
CGGTGCTACA GAGTTCTTGA AGTGGTGGCC TAACTACGGC TACACTAGAA GGACAGTATT

     13330      13340      13350      13360      13370      13380
TGGTATCTGC GCTCTGCTGA AGCCAGTTAC CTTCGGAAAA AGAGTTGGTA GCTCTTGATC

     13390      13400      13410      13420      13430      13440
  CAAACAA ACCACCGCTG GTAGCGGTGG TTTTTTTGTT TGCAAGCAGC AGATTACGCG

     13450      13460      13470      13480      13490      13500
CAGAAAAAAA GGATCTCAAG AAGATCCTTT GATCTTTTCT ACGGGGTCTG ACGCTCAGTG

     13510      13520      13530      13540      13550      13560
GAACGAAAAC TCACGTTAAG GGATTTTGGT CATGAGATTA TCAAAAAGGA TCTTCACCTA

     13570      13580      13590      13600      13610      13620
GATCCTTTTA AATTAAAAAT GAAGTTTTAA ATCAATCTAA AGTATATATG AGTAAACTTG

     13630      13640      13650      13660      13670      13680
GTCTGACAGT TACCAATGCT TAATCAGTGA GGCACCTATC TCAGCGATCT GTCTATTTCG

     13690      13700      13710      13720      13730      13740
TTCATCCATA GTTGCCTGAC TCCCCGTCGT GTAGATAACT ACGATACGGG AGGGCTTACC

     13750      13760      13770      13780      13790      13800
  CTGGCCCC AGTGCTGCAA TGATACCGCG AGACCCACGC TCACCGGCTC CAGATTTATC

     13810      13820      13830      13840      13850      13860
AGCAATAAAC CAGCCAGCCG GAAGGGCCGA GCGCAGAAGT GGTCCTGCAA CTTTATCCGC

     13870      13880      13890      13900      13910      13920
CTCCATCCAG TCTATTAATT GTTGCCGGGA AGCTAGAGTA AGTAGTTCGC CAGTTAATAG

     13930      13940      13950      13960      13970      13980
TTTGCGCAAC GTTGTTGCCA TTGCTGCAGG CATCGTGGTG TCACGCTCGT CGTTTGGTAT

     13990      14000      14010      14020      14030      14040
GGCTTCATTC AGCTCCGGTT CCCAACGATC AAGGCGAGTT ACATGATCCC CCATGTTGTG

     14050      14060      14070      14080      14090      14100
CAAAAAAGCG GTTAGCTCCT TCGGTCCTCC GATCGTTGTC AGAAGTAAGT TGGCCGCAGT

     14110      14120      14130      14140      14150      14160
GTTATCACTC ATGGTTATGG CAGCACTGCA TAATTCTCTT ACTGTCATGC CATCCGTAAG

     14170      14180      14190      14200      14210      14220
ATGCTTTTCT GTGACTGGTG AGTACTCAAC CAAGTCATTC TGAGAATAGT GTATGCGGCG

     14230      14240      14250      14260      14270      14280
ACCGAGTTGC TCTTGCCCGG CGTCAACACG GGATAATACC GCGCCACATA GCAGAACTTT

     14290      14300      14310      14320      14330      14340
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DNASIS
Desmond Lark

AAAAGTGCTC ATCATTGGAA AACGTTCTTC GGGGCGAAAA CTCTCAAGGA TCTTACCGCT

14350 14360 14370 14380 14390 14400
GTTGAGATCC AGTTCGATGT AACCCACTCG TGCACCCAAC TGATCTTCAG CATCTTTTAC

14410 14420 14430 14440 14450 14460
TTTCACCAGC GTTTCTGGGT GAGCAAAAAC AGGAAGGCAA AATGCCGCAA AAAAGGGAAT

14470 14480 14490 14500 14510 14520
AAGGGCGACA CGGAAATGTT GAATACTCAT ACTCTTCCTT TTTCAATATT ATTGAAGCAT

14530 14540 14550 14560 14570 14580
TTATCAGGGT TATTGTCTCA TGAGCGGATA CATATTTGAA TGTATTTAGA AAAATAAACA

14590 14600 14610 14620 14630 14640
AATAGGGGTT CCGCGCACAT TTCCCCGAAA AGTGCCACCT GACGTCTAAG AAACCATTAT

14650 14660 14670 14680 14690 14700
TATCATGACA TTAACCTATA AAAATAGGCG TATCACGAGG CCCTTTCGTC TTCAAGAA..

FIGURE 8

2/3/98

DNASIS
Holly Lark

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        10         20         30         40         50         60
TTAATTAAGG GGCGGAGAAT GGGCGGAACT GGGCGGAGTT AGGGGCGGGA TGGGCGGAGT

        70         80         90        100        110        120
TAGGGGCGGG ACTATGGTTG CTGACTAATT GAGATGCATG CTTTGCATAC TTCTGCCTGC

       130        140        150        160        170        180
TGGGGAGCCT GGGGACTTTC CACACCTGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT

       190        200        210        220        230        240
ACTTCTGCCT GCTGGGGAGC CTGGGGACTT TCCACACCCT AACTGACACA CATTCCACAG

       250        260        270        280        290        300
AATTAATTCC CCTAGTTATT AATAGTAATC AATTACGGGG TCATTAGTTC ATAGCCCATA

       310        320        330        340        350        360
TATGGAGTTC CGCGTTACAT AACTTACGGT AAATGGCCCG CCTGGCTGAC CGCCCAACGA

       370        380        390        400        410        420
   CCGCCCA TTGACGTCAA TAATGACGTA TGTTCCCATA GTAACGCCAA TAGGGACTTT

       430        440        450        460        470        480
CCATTGACGT CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT

       490        500        510        520        530        540
GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAAATGGC CCGCCTGGCA

       550        560        570        580        590        600
TTATGCCCAG TACATGACCT TATGGGACTT TCCTACTTGG CAGTACATCT ACGTATTAGT

       610        620        630        640        650        660
CATCGCTATT ACCATGGTGA TGCGGTTTTG GCAGTACATC AATGGGCGTG GATAGCGGTT

       670        680        690        700        710        720
TGACTCACGG GGATTTCCAA GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGAAG

       730        740        750        760        770        780
  TGGCCGGC CAGCTTTATT TAACGTGTTT ACGTCGAGTC AATTGTACAC TAACGACAGT

       790        800        810        820        830        840
GATGAAAGAA ATACAAAAGC GCATAATATT TTGAACGACG TCGAACCTTT ATTACAAAAC

       850        860        870        880        890        900
AAAACACAAA CGAATATCGA CAAAGCTAGA TTGCTGCTAC AAGATTTGGC AAGTTTTGTG

       910        920        930        940        950        960
GCGTTGAGCG AAAATCCATT AGATAGTCCA GCCATCGGTT CGGAAAAACA ACCCTTGTTT

       970        980        990       1000       1010       1020
GAAACTAATC GAAACCTATT TTACAAATCT ATTGAGGATT TAATATTTAA ATTCAGATAT

      1030       1040       1050       1060       1070       1080
AAAGACGCTG AAAATCATTT GATTTTCGCT CTAACATACC ACCCTAAAGA TTATAAATTT

      1090       1100       1110       1120       1130       1140
AATGAATTAT TAAAATACAT CAGCAACTAT ATATTGATAG ACATTTCCAG TTTGTGATAT

      1150       1160       1170       1180       1190       1200
TAGTTTGTGC GTCTCATTAC AATGGCTGTT ATTTTTAACA ACAAACAACT GCTCGCAGAC

      1210       1220       1230       1240       1250       1260
AATAGTATAG AAAAGGGAGG TGAACTGTTT TTGTTTAACG GTTCGTACAA CATTTTGGAA

      1270       1280       1290       1300       1310       1320
AGTTATGTTA ATCCGGTGCT GCTAAAAAAT GGTGTAATTG AACTAGAAGA AGCTGCGTAC
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DNASIS
Molly. Lark

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      1330       1340       1350       1360       1370       1380
TATGCCGGCA ACATATTGTA CAAAACCGAC GATCCCAAAT TCATTGATTA TATAAATTTA

      1390       1400       1410       1420       1430       1440
ATAATTAAAG CAACACACTC CGAAGAACTA CCAGAAAATA GCACTGTTGT AAATTACAGA

      1450       1460       1470       1480       1490       1500
AAAACTATGC GCAGCGGTAC TATACACCCC ATTAAAAAAG ACATATATAT TTATGACAAC

      1510       1520       1530       1540       1550       1560
AAAAAATTTA CTCTATACGA TAGATACATA TATGGATACG ATAATAACTA TGTTAATTTT

      1570       1580       1590       1600       1610       1620
TATGAGGAGA AAAATGAAAA AGAGAAGGAA TACGAAGAAG AAGACGACAA GGCGTCTAGT

      1630       1640       1650       1660       1670       1680
TTATGTGAAA ATAAAATTAT ATTGTCGCAA ATTAACTGTG AATCATTTGA AAATGATTTT

      1690       1700       1710       1720       1730       1740
AAATATTACC TCAGCGATTA TAACTACGCG TTTTCAATTA TAGATAATAC TACAAATGTT

      1750       1760       1770       1780       1790       1800
CTTGTTGCGT TTGGTTTGTA TCGTTAATAA AAAACAAATT TGACATTTAT AATTGTTTTA

      1810       1820       1830       1840       1850       1860
TTATTCAATA ATTACAAATA GGATTGAGAC CCTTGCAGTT GCCAGCAAAC GGACAGAGCT

      1870       1880       1890       1900       1910       1920
TGTCGAGGAG AGTTGTTGAT TCATTGTTTG CCTCCCTGCT GCGGTTTTTC ACCGAAGTTC

      1930       1940       1950       1960       1970       1980
ATGCCAGTCC AGCGTTTTTG CAGCAGAAAA GCCGCCGACT TCGGTTTGCG GTCGCGAGTG

      1990       2000       2010       2020       2030       2040
AAGATCCCTT TCTTGTTACC GCCAACGCGC AATATGCCTT GCGAGGTCGC AAAATCGGCG

      2050       2060       2070       2080       2090       2100
AAATTCCATA CCTGTTCACC GACGACGGCG CTGACGCGAT CAAAGACGCG GTGATACATA

      2110       2120       2130       2140       2150       2160
TCCAGCCATG CACACTGATA CTCTTCACTC CACATGTCGG TGTACATTGA GTGCAGCCCG

      2170       2180       2190       2200       2210       2220
GCTAACGTAT CCACGCCGTA TTCGGTGATG ATAATCGGCT GATGCAGTTT CTCCTGCCAG

      2230       2240       2250       2260       2270       2280
GCCAGAAGTT CTTTTTCCAG TACCTTCTCT GCCGTTTCCA AATCGCCGCT TTGGACATAC

      2290       2300       2310       2320       2330       2340
CATCCGTAAT AACGGTTCAG GCACAGCACA TCAAAGAGAT CGCTGATGGT ATCGGTGTGA

      2350       2360       2370       2380       2390       2400
GCGTCGCAGA ACATTACATT GACGCAGGTG ATCGGACGCG TCGGGTCGAG TTTACGCGTT

      2410       2420       2430       2440       2450       2460
GCTTCCGCCA GTGGCGCGAA ATATTCCCGT GCACCTTGCG GACGGGTATC CGGTTCGTTG

      2470       2480       2490       2500       2510       2520
GCAATACTCC ACATCACCAC GCTTGGGTGG TTTTTGTCAC GCGCTATCAG CTCTTTAATC

      2530       2540       2550       2560       2570       2580
GCCTGTAAGT GCGCTTGCTG AGTTTCCCCG TTGACTGCCT CTTCGCTGTA CAGTTCTTTC

      2590       2600       2610       2620       2630       2640
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DNASIS
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GGCTTGTTGC CCGCTTCGAA ACCAATGCCT AAAGAGAGGT TAAAGCCGAC AGCAGCAGTT

      2650       2660       2670       2680       2690       2700
TCATCAATCA CCACGATGCC ATGTTCATCT GCCCAGTCGA GCATCTCTTC AGCGTAAGGG

      2710       2720       2730       2740       2750       2760
TAATGCGAGG TACGGTAGGA GTTGGCCCCA ATCCAGTCCA TTAATGCGTG GTCGTGCACC

      2770       2780       2790       2800       2810       2820
ATCAGCACGT TATCGAATCC TTTGCCACGC AAGTCCGCAT CTTCATGACG ACCAAAGCCA

      2830       2840       2850       2860       2870       2880
GTAAAGTAGA ACGGTTTGTG GTTAATCAGG AACTGTTCGC CCTTCACTGC CACTGACCGG

      2890       2900       2910       2920       2930       2940
ATGCCGACGC GAAGCGGGTA GATATCACAC TCTGTCTGGC TTTTGGCTGT GACGCACAGT

      2950       2960       2970       2980       2990       3000
--ATAGAGAT AACCTTCACC CGGTTGCCAG AGGTGCGGAT TCACCACTTG CAAAGTCCCG

      3010       3020       3030       3040       3050       3060
CTAGTGCCTT GTCCAGTTGC AACCACCTGT TGATCCGCAT CACGCAGTTC AACGCTGACA

      3070       3080       3090       3100       3110       3120
TCACCATTGG CCACCACCTG CCAGTCAACA GACGCGTGGT TACAGTCTTG CGCGACATGC

      3130       3140       3150       3160       3170       3180
GTCACCACGG TGATATCGTC CACCCAGGTG TTCGGCGTGG TGTAGAGCAT TACGCTGCGA

      3190       3200       3210       3220       3230       3240
TGGATTCCGG CATAGTTAAA GAAATCATGG AAGTAAGACT GCTTTTTCTT GCCGTTTTCG

      3250       3260       3270       3280       3290       3300
TCGGTAATCA CCATTCCCGG CGGGATAGTC TGCCAGTTCA GTTCGTTGTT CACACAAACG

      3310       3320       3330       3340       3350       3360
--GATACCCC TCGACGGATT AAAGACTTCA AGCGGTCAAC TATGAAGAAG TGTTCGTCTT

      3370       3380       3390       3400       3410       3420
CGTCCCAGTA AGCTATGTCT CCAGAATGTA GCCATCCATC CTTGTCAATC AAGGCGTTGG

      3430       3440       3450       3460       3470       3480
TCGCTTCCGG ATTGTTTACA TAACCGGACA TAATCATAGG TCCTCTGACA CATAATTCGC

      3490       3500       3510       3520       3530       3540
CTCTCTGATT AACGCCCAGC GTTTTCCCGG TATCCAGATC CACAACCTTC GCTTCAAAAA

      3550       3560       3570       3580       3590       3600
ATGGAACAAC TTTACCGACC GCGCCCGGTT TATCATCCCC CTCGGGTGTA ATCAGAATAG

      3610       3620       3630       3640       3650       3660
CTGATGTAGT CTCAGTGAGC CCATATCCTT GTCGTATCCC TGGAAGATGG AAGCGTTTTG

      3670       3680       3690       3700       3710       3720
CAACCGCTTC CCCGACTTCT TTCGAAAGAG GTGCGCCCCC AGAAGCAATT TCGTGTAAAT

      3730       3740       3750       3760       3770       3780
TAGATAAATC GTATTTGTCA ATCAGAGTGC TTTTGGCGAA GAATGAAAAT AGGGTTGGTA

      3790       3800       3810       3820       3830       3840
CTAGCAACGC ACTTTGAATT TTGTAATCCT GAAGGGATCG TAAAAACAGC TCTTCTTCAA

      3850       3860       3870       3880       3890       3900
ATCTATACAT TAAGACGACT CGAAATCCAC ATATCAAATA TCCGAGTGTA GTAAACATTC
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3910 3920 3930 3940 3950 3960
CAAAACCGTG ATGGAATGGA ACAACACTTA AAATCGCAGT ATCCGGAATG ATTTGATTGC

3970 3980 3990 4000 4010 4020
CAAAAATAGG ATCTCTGGCA TGCGAGAATC TGACGCAGGC AGTTCTATGC GGAAGGGCCA

4030 4040 4050 4060 4070 4080
CACCCTTAGG TAACCCAGTA GATCCAGAGG AATTGTTTTG TCACGATCAA AGGACTCTGG

4090 4100 4110 4120 4130 4140
TACAAAATCG TATTCATTAA AACCGGGAGG TAGATGAGAT GTGACGAACG TGTACATCGA

4150 4160 4170 4180 4190 4200
CTGAAATCCC TGGTAATCCG TTTTAGAATC CATGATAATA ATTTTCTGGA TTATTGGTAA

4210 4220 4230 4240 4250 4260
TTTTTTTTGC ACGTTCAAAA TTTTTTGCAA CCCCTTTTTG GAAACAAACA CTACGGTAGG

4270 4280 4290 4300 4310 4320
;CGAAATG TTCATACTGT TGAGCAATTC ACGTTCATTA TAAATGTCGT TCGCGGGCGC

4330 4340 4350 4360 4370 4380
AACTGCAACT CCGATAAATA ACGCGCCCAA CACCGGCATA AAGAATTGAA GAGAGTTTTC

4390 4400 4410 4420 4430 4440
ACTGCATACG ACGATTCTGT GATTTGTATT CAGCCCATAT CGTTTCATAG CTTCTGCCAA

4450 4460 4470 4480 4490 4500
CCGAACGGAC ATTTCGAAGT ATTCCGCGTA CGTGATGTTC ACCTCGATAT GTGCATCTGT

4510 4520 4530 4540 4550 4560
AAAAGGAATT GTTCCAGGAA CCAGGGCGTA TCTCTTCATA GCCTTATGCA GTTGCTCTCC

4570 4580 4590 4600 4610 4620
AGCGGTTCCA TCCTCTAGCT TTGCTTCTCA ATTTCTTATT TGCATAATGA GAAAAAAAGG

4630 4640 4650 4660 4670 4680
\ATTAATT TTAACACCAA TTCAGTAGTT GATTGAGCAA ATGCGTTGCC AAAAAGGATG

4690 4700 4710 4720 4730 4740
CTTTAGAGAC AGTGTTCTCT GCACAGATAA GGACAAACAT TATTCAGAGG GAGTACCCAG

4750 4760 4770 4780 4790 4800
AGCTGAGACT CCTAAGCCAG TGAGTGGCAC AGCATCCAGG GAGAAATATG CTTGTCATCA

4810 4820 4830 4840 4850 4860
CCGAAGCCTG ATTCCGTAGA GCCACACCCT GGTAAGGGCC AATCTGCTCA CACAGGATAG

4870 4880 4890 4900 4910 4920
AGAGGGCAGG AGCCAGGGCA GAGCATATAA GGTGAGGTAG GATCAGTTGC TCCTCACATT

4930 4940 4950 4960 4970 4980
TGCTTCTGAC ATAGTTGTGT TGGGAGCTTG GATCGATCCA CCATGGGCTT CAATACCCTG

4990 5000 5010 5020 5030 5040
ATTGACTGGA ACAGCTGTAG CCCTGAACAG CAGCGTGCGC TGCTGACGCG TCCGGCGATT

5050 5060 5070 5080 5090 5100
TCCGCCTCTG ACAGTATTAC CCGGACGGTC AGCGATATTC TGGATAATGT AAAAACGCGC

5110 5120 5130 5140 5150 5160
GGTGACGATG CCCTGCGTGA.ATACAGCGCT AAATTTGATA AAACAGAAGT GACAGCGCTA

5170 5180 5190 5200 5210 5220
CGCGTCACCC CTGAAGAGAT CGCCGCCGCC GGCGCGCGTC TGAGCGACGA ATTAAAACAG

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      5230       5240       5250       5260       5270       5280
GCGATGACCG CTGCCGTCAA AAATATTGAA ACGTTCCATT CCGCGCAGAC GCTACCGCCT

      5290       5300       5310       5320       5330       5340
GTAGATGTGG AAACCCAGCC AGGCGTGCGT TGCCAGCAGG TTACGCGTCC CGTCTCGTCT

      5350       5360       5370       5380       5390       5400
GTCGGTCTGT ATATTCCCGG CGGCTCGGCT CCGCTCTTCT CAACGGTGCT GATGCTGGCG

      5410       5420       5430       5440       5450       5460
ACGCCGGCGC GCATTGCGGG ATGCCAGAAG GTGGTTCTGT GCTCGCCGCC GCCCATCGCT

      5470       5480       5490       5500       5510       5520
GATGAAATCC TCTATGCGGC GCAACTGTGT GGCGTGCAGG AAATCTTTAA CGTCGGCGGC

      5530       5540       5550       5560       5570       5580
GCGCAGGCGA TTGCCGCTCT GGCCTTCGGC AGCGAGTCCG TACCGAAAGT GGATAAAATT

      5590       5600       5610       5620       5630       5640
.TGGCCCCG GCAACGCCTT TGTAACCGAA GCCAAACGTC AGGTCAGCCA GCGTCTCGAC

      5650       5660       5670       5680       5690       5700
GGCGCGGCTA TCGATATGCC AGCCGGGCCG TCTGAAGTAC TGGTGATCGC AGACAGCGGC

      5710       5720       5730       5740       5750       5760
GCAACACCGG ATTTCGTCGC TTCTGACCTG CTCTCCCAGG CTGAGCACGG CCCGGATTCC

      5770       5780       5790       5800       5810       5820
CAGGTGATCC TGCTGACGCC TGATGCTGAC ATTGCCCGCA AGGTGGCGGA GGCGGTAGAA

      5830       5840       5850       5860       5870       5880
CGTCAACTGG CGGAACTGCC GCGCGCGGAC ACCGCCCGGC AGGCCCTGAG CGCCAGTCGT

      5890       5900       5910       5920       5930       5940
CTGATTGTGA CCAAAGATTT AGCGCAGTGC GTCGCCATCT CTAATCAGTA TGGGCCGGAA

      5950       5960       5970       5980       5990       6000
ACTTAATCA TCCAGACGCG CAATGCGCGC GATTTGGTGG ATGCGATTAC CAGCGCAGGC

      6010       6020       6030       6040       6050       6060
TCGGTATTTC TCGGCGACTG GTCGCCGGAA TCCGCCGGTG ATTACGCTTC CGGAACCAAC

      6070       6080       6090       6100       6110       6120
CATGTTTTAC CGACCTATGG CTATACTGCT ACCTGTTCCA GCCTTGGGTT AGCGGATTTC

      6130       6140       6150       6160       6170       6180
CAGAAACGGA TGACCGTTCA GGAACTGTCG AAAGCGGGCT TTTCCGCTCT GGCATCAACC

      6190       6200       6210       6220       6230       6240
ATTGAAACAT TGGCGGCGGC AGAACGTCTG ACCGCCCATA AAAATGCCGT GACCCTGCGC

      6250       6260       6270       6280       6290       6300
GTAAACGCCC TCAAGGAGCA AGCATGAGGC ACTGAAAACA CTCTCAGCGT CGCTGACTTA

      6310       6320       6330       6340       6350       6360
GCCCGTGAAA ATGTCCGCAA CCTGGAGATC CAGACATGAT AAGATACATT GATGAGTTTG

      6370       6380       6390       6400       6410       6420
GACAAACCAC AACTAGAATG CAGTGAAAAA AATGCTTTAT TTGTGAAATT TGTGATGCTA

      6430       6440       6450       6460       6470       6480
TTGCTTTATT TGTAACCATT ATAAGCTGCA ATAAACAAGT TAACAACAAC AATTGCATTC

      6490       6500       6510       6520       6530       6540
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ATTTTATGTT TCAGGTTCAG GGGGAGGTGT GGGAGGTTTT TTAAAGCAAG TAAAACCTCT

6550 6560 6570 6580 6590 -6600
ACAAATGTGG TATGGCTGAT TATGATCTCT AGGGCCGGCC CTCGACGGCG CGCCTCTAGA

6610 6620 6630 6640 6650 6660
GCAGTGTGGT TTTGCAAGAG GAAGCAAAAA GCCTCTCCAC CCAGGCCTGG AATGTTTCCA

6670 6680 6690 6700 6710 6720
CCCAATGTCG AGCAGTGTGG TTTTGCAAGA GGAAGCAAAA AGCCTCTCCA CCCAGGCCTG

6730 6740 6750 6760 6770 6780
GAATGTTTCC ACCCAATGTC GAGCAAACCC CGCCCAGCGT CTTGTCATTG GCGAATTCGA

6790 6800 6810 6820 6830 6840
ACACGCAGAT GCAGTCGGGG CGGCGCGGTC CCAGGTCCAC TTCGCATATT AAGGTGACGC

6850 6860 6870 6880 6890 6900
CTGTGGCCTC GAACACCGAG CGACCCTGCA GCCAATATGG GATCGGCCAT TGAACAAGAT

6910 6920 6930 6940 6950 6960
GGATTGCACG CAGGTTCTCC GGCCGCTTGG GTGGAGAGGC TATTCGGCTA TGACTGGGCA

6970 6980 6990 7000 7010 7020
CAACAGACAA TCGGCTGCTC TGATGCCGCC GTGTTCCGGC TGTCAGCGCA GGGGCGCCCG

7030 7040 7050 7060 7070 7080
GTTCTTTTTG TCAAGACCGA CCTGTCCGGT GCCCTGAATG AACTGCAGGT AAGTGCGGCC

7090 7100 7110 7120 7130 7140
GTCGATGGCC GAGGCGGCCT CGGCCTCTGC ATAAATAAAA AAAATTAGTC AGCCATGCAT

7150 7160 7170 7180 7190 7200
GGGGCGGAGA ATGGGCGGAA CTGGGCGGAG TTAGGGGCGG GATGGGCGGA GTTAGGGGCG

7210 7220 7230 7240 7250 7260
GGACTATGGT TGCTGACTAA TTGAGATGCA TGCTTTGCAT ACTTCTGCCT GCTGGGGAGC

7270 7280 7290 7300 7310 7320
CTGGGGACTT TCCACACCTG GTTGCTGACT AATTGAGATG CATGCTTTGC ATACTTCTGC

7330 7340 7350 7360 7370 7380
CTGCTGGGGA GCCTGGGGAC TTTCCACACC CTAACTGACA CACATTCCAC AGAATTAATT

7390 7400 7410 7420 7430 7440
CCCCTAGTTA TTAATAGTAA TCAATTACGG GGTCATTAGT TCATAGCCCA TATATGGAGT

7450 7460 7470 7480 7490 7500
TCCGCGTTAC ATAACTTACG GTAAATGGCC CGCCTGGCTG ACCGCCCAAC GACCCCCGCC

7510 7520 7530 7540 7550 7560
CATTGACGTC AATAATGACG TATGTTCCCA TAGTAACGCC AATAGGGACT TTCCATTGAC

7570 7580 7590 7600 7610 7620
GTCAATGGGT GGACTATTTA CGGTAAACTG CCCACTTGGC AGTACATCAA GTGTATCATA

7630 7640 7650 7660 7670 7680
TGCCAAGTAC GCCCCCTATT GACGTCAATG ACGGTAAATG GCCCGCCTGG CATTATGCCC

7690 7700 7710 7720 7730 7740
AGTACATGAC CTTATGGGAC TTTCCTACTT GGCAGTACAT CTACGTATTA GTCATCGCTA

7750 7760 7770 7780 7790 7800
TTACCATGGT GATGCGGTTT TGGCAGTACA TCAATGGGCG TGGATAGCGG TTTGACTCAC

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7810	7820	7830	7840	7850	7860
GGGGATTTCC	AAGTCTCCAC	CCCATTGACG	TCAATGGGAG	TTTGTTTTGG	CACCAAAATC
7870	**7880**	**7890**	**7900**	**7910**	**7920**
AACGGGACTT	TCCAAAATGT	CGTAACAACT	CCGCCCCATT	GACGCAAATG	GGCGGTAGGC
7930	**7940**	**7950**	**7960**	**7970**	**7980**
GTGTACGGTG	GGAGGTCTAT	ATAAGCAGAG	CTGGGTACGT	GAACCGTCAG	ATCGCCTGGA
7990	**8000**	**8010**	**8020**	**8030**	**8040**
GACGCCATCA	CAGATCTCTC	ACTATGGATT	TTCAGGTGCA	GATTATCAGC	TTCCTGCTAA
8050	**8060**	**8070**	**8080**	**8090**	**8100**
TCAGTGCTTC	AGTCATAATG	TCCAGAGGAC	AAATTGTTCT	CTCCCAGTCT	CCAGCAATCC
8110	**8120**	**8130**	**8140**	**8150**	**8160**
TGTCTGCATC	TCCAGGGGAG	AAGGTCACAA	TGACTTGCAG	GGCCAGCTCA	AGTGTAAGTT
8170	**8180**	**8190**	**8200**	**8210**	**8220**
ATCCACTG	GTTCCAGCAG	AAGCCAGGAT	CCTCCCCCAA	ACCCTGGATT	TATGCCACAT
8230	**8240**	**8250**	**8260**	**8270**	**8280**
CCAACCTGGC	TTCTGGAGTC	CCTGTTCGCT	TCAGTGGCAG	TGGGTCTGGG	ACTTCTTACT
8290	**8300**	**8310**	**8320**	**8330**	**8340**
CTCTCACAAT	CAGCAGAGTG	GAGGCTGAAG	ATGCTGCCAC	TTATTACTGC	CAGCAGTGGA
8350	**8360**	**8370**	**8380**	**8390**	**8400**
CTAGTAACCC	ACCCACGTTC	GGAGGGGGGA	CCAAGCTGGA	AATCAAACGT	ACGGTGGCTG
8410	**8420**	**8430**	**8440**	**8450**	**8460**
CACCATCTGT	CTTCATCTTC	CCGCCATCTG	ATGAGCAGTT	GAAATCTGGA	ACTGCCTCTG
8470	**8480**	**8490**	**8500**	**8510**	**8520**
TTGTGTGCCT	GCTGAATAAC	TTCTATCCCA	GAGAGGCCAA	AGTACAGTGG	AAGGTGGATA
8530	**8540**	**8550**	**8560**	**8570**	**8580**
GCCCTCCA	ATCGGGTAAC	TCCCAGGAGA	GTGTCACAGA	GCAGGACAGC	AAGGACAGCA
8590	**8600**	**8610**	**8620**	**8630**	**8640**
CCTACAGCCT	CAGCAGCACC	CTGACGCTGA	GCAAAGCAGA	CTACGAGAAA	CACAAAGTCT
8650	**8660**	**8670**	**8680**	**8690**	**8700**
ACGCCTGCGA	AGTCACCCAT	CAGGGCCTGA	GCTCGCCCGT	CACAAAGAGC	TTCAACAGGG
8710	**8720**	**8730**	**8740**	**8750**	**8760**
GAGAGTGTTG	AATTCAGATC	CGTTAACGGT	TACCAACTAC	CTAGACTGGA	TTCGTGACAA
8770	**8780**	**8790**	**8800**	**8810**	**8820**
CATGCGGCCG	TGATATCTAC	GTATGATCAG	CCTCGACTGT	GCCTTCTAGT	TGCCAGCCAT
8830	**8840**	**8850**	**8860**	**8870**	**8880**
CTGTTGTTTG	CCCCTCCCCC	GTGCCTTCCT	TGACCCTGGA	AGGTGCCACT	CCCACTGTCC
8890	**8900**	**8910**	**8920**	**8930**	**8940**
TTTCCTAATA	AAATGAGGAA	ATTGCATCGC	ATTGTCTGAG	TAGGTGTCAT	TCTATTCTGG
8950	**8960**	**8970**	**8980**	**8990**	**9000**
GGGGTGGGGT	GGGGCAGGAC	AGCAAGGGGG	AGGATTGGGA	AGACAATAGC	AGGCATGCTG
9010	**9020**	**9030**	**9040**	**9050**	**9060**
GGGATGCGGT	GGGCTCTATG	GAACCAGCTG	GGGCTCGACA	GCTATGCCAA	GTACGCCCCC
9070	**9080**	**9090**	**9100**	**9110**	**9120**
TATTGACGTC	AATGACGGTA	AATGGCCCGC	CTGGCATTAT	GCCCAGTACA	TGACCTTATG

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      9130       9140       9150       9160       9170       9180
GGACTTTCCT ACTTGGCAGT ACATCTACGT ATTAGTCATC GCTATTACCA TGGTGATGCG

      9190       9200       9210       9220       9230       9240
GTTTTGGCAG TACATCAATG GGCGTGGATA GCGGTTTGAC TCACGGGGAT TTCCAAGTCT

      9250       9260       9270       9280       9290       9300
CCACCCCATT GACGTCAATG GGAGTTTGTT TTGGCACCAA AATCAACGGG ACTTTCCAAA

      9310       9320       9330       9340       9350       9360
ATGTCGTAAC AACTCCGCCC CATTGACGCA AATGGGCGGT AGGCGTGTAC GGTGGGAGGT

      9370       9380       9390       9400       9410       9420
CTATATAAGC AGAGCTGGGT ACGTCCTCAC ATTCAGTGAT CAGCACTGAA CACAGACCCG

      9430       9440       9450       9460       9470       9480
TCGACATGGG TTGGAGCCTC ATCTTGCTCT TCCTTGTCGC TGTTGCTACG CGTGTCCTGT

      9490       9500       9510       9520       9530       9540
CCCAGGTACA ACTGCAGCAG CCTGGGGCTG AGCTGGTGAA GCCTGGGGCC TCAGTGAAGA

      9550       9560       9570       9580       9590       9600
TGTCCTGCAA GGCTTCTGGC TACACATTTA CCAGTTACAA TATGCACTGG GTAAAACAGA

      9610       9620       9630       9640       9650       9660
CACCTGGTCG GGGCCTGGAA TGGATTGGAG CTATTTATCC CGGAAATGGT GATACTTCCT

      9670       9680       9690       9700       9710       9720
ACAATCAGAA GTTCAAAGGC AAGGCCACAT TGACTGCAGA CAAATCCTCC AGCACAGCCT

      9730       9740       9750       9760       9770       9780
ACATGCAGCT CAGCAGCCTG ACATCTGAGG ACTCTGCGGT CTATTACTGT GCAAGATCGA

      9790       9800       9810       9820       9830       9840
CTTACTACGG CGGTGACTGG TACTTCAATG TCTGGGGCGC AGGGACCACG GTCACCGTCT

      9850       9860       9870       9880       9890       9900
CTGCAGCTAG CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT

      9910       9920       9930       9940       9950       9960
CTGGGGGCAC AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG

      9970       9980       9990      10000      10010      10020
TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT GTCCTACAGT

     10030      10040      10050      10060      10070      10080
CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC TTGGGCACCC

     10090      10100      10110      10120      10130      10140
AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC AAGAAAGCAG

     10150      10160      10170      10180      10190      10200
AGCCCAAATC TTGTGACAAA ACTCACACAT GCCCACCGTG CCCAGCACCT GAACTCCTGG

     10210      10220      10230      10240      10250      10260
GGGGACCGTC AGTCTTCCTC TTCCCCCCAA AACCCAAGGA CACCCTCATG ATCTCCCGGA

     10270      10280      10290      10300      10310      10320
CCCCTGAGGT CACATGCGTG GTGGTGGACG TGAGCCACGA AGACCCTGAG GTCAAGTTCA

     10330      10340      10350      10360      10370      10380
ACTGGTACGT GGACGGCGTG GAGGTGCATA ATGCCAAGAC AAAGCCGCGG GAGGAGCAGT

     10390      10400      10410      10420      10430      10440
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ACAACAGCAC GTACCGTGTG GTCAGCGTCC TCACCGTCCT GCACCAGGAC TGGCTGAATG

10450 10460 10470 10480 10490 10500
GCAAGGAGTA CAAGTGCAAG GTCTCCAACA AAGCCCTCCC AGCCCCCATC GAGAAAACCA

10510 10520 10530 10540 10550 10560
TCTCCAAAGC CAAAGGGCAG CCCCGAGAAC CACAGGTGTA CACCCTGCCC CCATCCCGGG

10570 10580 10590 10600 10610 10620
ATGAGCTGAC CAAGAACCAG GTCAGCCTGA CCTGCCTGGT CAAAGGCTTC TATCCCAGCG

10630 10640 10650 10660 10670 10680
ACATCGCCGT GGAGTGGGAG AGCAATGGGC AGCCGGAGAA CAACTACAAG ACCACGCCTC

10690 10700 10710 10720 10730 10740
CCGTGCTGGA CTCCGACGGC TCCTTCTTCC TCTACAGCAA GCTCACCGTG GACAAGAGCA

10750 10760 10770 10780 10790 10800
GGTGGCAGCA GGGGAACGTC TTCTCATGCT CCGTGATGCA TGAGGCTCTG CACAACCACT

10810 10820 10830 10840 10850 10860
ACACGCAGAA GAGCCTCTCC CTGTCTCCGG GTAAATGAGG ATCCGTTAAC GGTTACCAAC

10870 10880 10890 10900 10910 10920
TACCTAGACT GGATTCGTGA CAACATGCGG CCGTGATATC TACGTATGAT CAGCCTCGAC

10930 10940 10950 10960 10970 10980
TGTGCCTTCT AGTTGCCAGC CATCTGTTGT TTGCCCCTCC CCCGTGCCTT CCTTGACCCT

10990 11000 11010 11020 11030 11040
GGAAGGTGCC ACTCCCACTG TCCTTTCCTA ATAAAATGAG GAAATTGCAT CGCATTGTCT

11050 11060 11070 11080 11090 11100
GAGTAGGTGT CATTCTATTC TGGGGGGTGG GGTGGGGCAG GACAGCAAGG GGGAGGATTG

11110 11120 11130 11140 11150 11160
GGAAGACAAT AGCAGGCATG CTGGGGATGC GGTGGGCTCT ATGGAACCAG CTGGGGCTCG

11170 11180 11190 11200 11210 11220
ACAGCAACGC TAGGTCGAGG CCGCTACTAA CTCTCTCCTC CCTCCTTTTT CCTGCAGGAC

11230 11240 11250 11260 11270 11280
GAGGCAGCGC GGCTATCGTG GCTGGCCACG ACGGGCGTTC CTTGCGCAGC TGTGCTCGAC

11290 11300 11310 11320 11330 11340
GTTGTCACTG AAGCGGGAAG GGACTGGCTG CTATTGGGCG AAGTGCCGGG GCAGGATCTC

11350 11360 11370 11380 11390 11400
CTGTCATCTC ACCTTGCTCC TGCCGAGAAA GTATCCATCA TGGCTGATGC AATGCGGCGG

11410 11420 11430 11440 11450 11460
CTGCATACGC TTGATCCGGC TACCTGCCCA TTCGACCACC AAGCGAAACA TCGCATCGAG

11470 11480 11490 11500 11510 11520
CGAGCACGTA CTCGGATGGA AGCCGGTCTT GTCGATCAGG ATGATCTGGA CGAAGAGCAT

11530 11540 11550 11560 11570 11580
CAGGGGCTCG CGCCAGCCGA ACTGTTCGCC AGGTAAGTGA GCTCCAATTC AAGCTTCCTA

11590 11600 11610 11620 11630 11640
GGGCGGCCAG CTAGTAGCTT TGCTTCTCAA TTTCTTATTT GCATAATGAG AAAAAAAGGA

11650 11660 11670 11680 11690 11700
AAATTAATTT TAACACCAAT TCAGTAGTTG ATTGAGCAAA TGCGTTGCCA AAAAGGATGC

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     11710      11720      11730      11740      11750      11760
TTTAGAGACA GTGTTCTCTG CACAGATAAG GACAAACATT ATTCAGAGGG AGTACCCAGA

     11770      11780      11790      11800      11810      11820
GCTGAGACTC CTAAGCCAGT GAGTGGCACA GCATCCAGGG AGAAATATGC TTGTCATCAC

     11830      11840      11850      11860      11870      11880
CGAAGCCTGA TTCCGTAGAG CCACACCCTG GTAAGGGCCA ATCTGCTCAC ACAGGATAGA

     11890      11900      11910      11920      11930      11940
GAGGGCAGGA GCCAGGGCAG AGCATATAAG GTGAGGTAGG ATCAGTTGCT CCTCACATTT

     11950      11960      11970      11980      11990      12000
GCTTCTGACA TAGTTGTGTT GGGAGCTTGG ATAGCTTGGG GGGGGGACAG CTCAGGGCTG

     12010      12020      12030      12040      12050      12060
CGATTTCGCG CCAAACTTGA CGGCAATCCT AGCGTGAAGG CTGGTAGGAT TTTATCCCCG

     12070      12080      12090      12100      12110      12120
  GCCATCAT GGTTCGACCA TTGAACTGCA TCGTCGCCGT GTCCCAAAAT ATGGGGATTG

     12130      12140      12150      12160      12170      12180
GCAAGAACGG AGACCTACCC TGGCCTCCGC TCAGGAACGA GTTCAAGTAC TTCCAAAGAA

     12190      12200      12210      12220      12230      12240
TGACCACAAC CTCTTCAGTG GAAGGTAAAC AGAATCTGGT GATTATGGGT AGGAAAACCT

     12250      12260      12270      12280      12290      12300
GGTTCTCCAT TCCTGAGAAG AATCGACCTT TAAAGGACAG AATTAATATA GTTCTCAGTA

     12310      12320      12330      12340      12350      12360
GAGAACTCAA AGAACCACCA CGAGGAGCTC ATTTTCTTGC CAAAAGTTTG GATGATGCCT

     12370      12380      12390      12400      12410      12420
TAAGACTTAT TGAACAACCG GAATTGGCAA GTAAAGTAGA CATGGTTTGG ATAGTCGGAG

     12430      12440      12450      12460      12470      12480
  AGTTCTGT TTACCAGGAA GCCATGAATC AACCAGGCCA CCTCAGACTC TTTGTGACAA

     12490      12500      12510      12520      12530      12540
GGATCATGCA GGAATTTGAA AGTGACACGT TTTTCCCAGA AATTGATTTG GGGAAATATA

     12550      12560      12570      12580      12590      12600
AACTTCTCCC AGAATACCCA GGCGTCCTCT CTGAGGTCCA GGAGGAAAAA GGCATCAAGT

     12610      12620      12630      12640      12650      12660
ATAAGTTTGA AGTCTACGAG AAGAAAGACT AACAGGAAGA TGCTTTCAAG TTCTCTGCTC

     12670      12680      12690      12700      12710      12720
CCCTCCTAAA GCTATGCATT TTTATAAGAC CATGGGACTT TTGCTGGCTT TAGATCAGCC

     12730      12740      12750      12760      12770      12780
TCGACTGTGC CTTCTAGTTG CCAGCCATCT GTTGTTTGCC CCTCCCCCGT GCCTTCCTTG

     12790      12800      12810      12820      12830      12840
ACCCTGGAAG GTGCCACTCC CACTGTCCTT TCCTAATAAA ATGAGGAAAT TGCATCGCAT

     12850      12860      12870      12880      12890      12900
TGTCTGAGTA GGTGTCATTC TATTCTGGGG GGTGGGGTGG GGCAGGACAG CAAGGGGGAG

     12910      12920      12930      12940      12950      12960
GATTGGGAAG ACAATAGCAG GCATGCTGGG GATGCGGTGG GCTCTATGGC TTCTGAGGCG

     12970      12980      12990      13000      13010      13020
GAAAGAACCA GCTGGGGCTC GAAGCGGCCG CCCATTTCGC TGGTGGTCAG ATGCGGGATG
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DNASIS
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      13030      13040      13050      13060      13070      13080
GCGTGGGACG CGGCGGGGAG CGTCACACTG AGGTTTTCCG CCAGACGCCA CTGCTGCCAG

      13090      13100      13110      13120      13130      13140
GCGCTGATGT GCCCGGCTTC TGACCATGCG GTCGCGTTCG GTTGCACTAC GCGTACTGTG

      13150      13160      13170      13180      13190      13200
AGCCAGAGTT GCCCGGCGCT CTCCGGCTGC GGTAGTTCAG GCAGTTCAAT CAACTGTTTA

      13210      13220      13230      13240      13250      13260
CCTTGTGGAG CGACATCCAG AGGCACTTCA CCGCTTGCCA GCGGCTTACC ATCCAGCGCC

      13270      13280      13290      13300      13310      13320
ACCATCCAGT GCAGGAGCTC GTTATCGCTA TGACGGAACA GGTATTCGCT GGTCACTTCG

      13330      13340      13350      13360      13370      13380
ATGGTTTGCC CGGATAAACG GAACTGGAAA AACTGCTGCT GGTGTTTTGC TTCCGTCAGC

      13390      13400      13410      13420      13430      13440
  .GGATGCG GCGTGCGGTC GGCAAAGACC AGACCGTTCA TACAGAACTG GCGATCGTTC

      13450      13460      13470      13480      13490      13500
GGCGTATCGC CAAAATCACC GCCGTAAGCC GACCACGGGT TGCCGTTTTC ATCATATTTA

      13510      13520      13530      13540      13550      13560
ATCAGCGACT GATCCACCCA GTCCCAGACG AAGCCGCCCT GTAAACGGGG ATACTGACGA

      13570      13580      13590      13600      13610      13620
AACGCCTGCC AGTATTTAGC GAAACCGCCA AGACTGTTAC CCATCGCGTG GGCGTATTCG

      13630      13640      13650      13660      13670      13680
CAAAGGATCA GCGGGCGCGT CTCTCCAGGT AGCGAAAGCC ATTTTTTGAT GGACCATTTC

      13690      13700      13710      13720      13730      13740
GGCACAGCCG GGAAGGGCTG GTCTTCATCC ACGCGCGCGT ACATCGGGCA AATAATATCG

      13750      13760      13770      13780      13790      13800
 .GGCCGTGG TGTCGGCTCC GCCGCCTTCA TACTGCACCG GGCGGGAAGG ATCGACAGAT

      13810      13820      13830      13840      13850      13860
TTGATCCAGC GATACAGCGC GTCGTGATTA GCGCCGTGGC CTGATTCATT CCCCAGCGAC

      13870      13880      13890      13900      13910      13920
CAGATGATCA CACTCGGGTG ATTACGATCG CGCTGCACCA TTCGCGTTAC GCGTTCGCTC

      13930      13940      13950      13960      13970      13980
ATCGCCGGTA GCCAGCGCGG ATCATCGGTC AGACGATTCA TTGGCACCAT GCCGTGGGTT

      13990      14000      14010      14020      14030      14040
TCAATATTGG CTTCATCCAC CACATACAGG CCGTAGCGGT CGCACAGCGT GTACCACAGC

      14050      14060      14070      14080      14090      14100
GGATGGTTCG GATAATGCGA ACAGCGCACG GCGTTAAAGT TGTTCTGCTT CATCAGCAGG

      14110      14120      14130      14140      14150      14160
ATATCCTGCA CCATCGTCTG CTCATCCATG ACCTGACCAT GCAGAGGATG ATGCTCGTGA

      14170      14180      14190      14200      14210      14220
CGGTTAACGC CTCGAATCAG CAACGGCTTG CCGTTCAGCA GCAGCAGACC ATTTTCAATC

      14230      14240      14250      14260      14270      14280
CGCACCTCGC GGAAACCGAC ATCGCAGGCT TCTGCTTCAA TCAGCGTGCC GTCGGCGGTG

      14290      14300      14310      14320      14330      14340
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DNASIS
Molly Lark

TGCAGTTCAA CCACCGCACG ATAGAGATTC GGGATTTCGG CGCTCCACAG TTTCGGGTTT

14350 14360 14370 14380 14390 14400
TCGACGTTCA GACGTAGTGT GACGCGATCG GCATAACCAC CACGCTCATC GATAATTTCA

14410 14420 14430 14440 14450 14460
CCGCCGAAAG GCGCGGTGCC GCTGGCGACC TGCGTTTCAC CCTGCCATAA AGAAACTGTT

14470 14480 14490 14500 14510 14520
ACCCGTAGGT AGTCACGCAA CTCGCCGCAC ATCTGAACTT CAGCCTCCAG TACAGCGCGG

14530 14540 14550 14560 14570 14580
CTGAAATCAT CATTAAAGCG AGTGGCAACA TGGAAATCGC TGATTTGTGT AGTCGGTTTA

14590 14600 14610 14620 14630 14640
TGCAGCAACG AGACGTCACG GAAAATGCCG CTCATCCGCC ACATATCCTG ATCTTCCAGA

14650 14660 14670 14680 14690 14700
TAACTGCCGT CACTCCAGCG CAGCACCATC ACCGCGAGGC GGTTTTCTCC GGCGCGTAAA

14710 14720 14730 14740 14750 14760
AATGCGCTCA GGTCAAATTC AGACGGCAAA CGACTGTCCT GGCCGTAACC GACCCAGCGC

14770 14780 14790 14800 14810 14820
CCGTTGCACC ACAGATGAAA CGCCGAGTTA ACGCCATCAA AAATAATTCG CGTCTGGCCT

14830 14840 14850 14860 14870 14880
TCCTGTAGCC AGCTTTCATC AACATTAAAT GTGAGCGAGT AACAACCCGT CGGATTCTCC

14890 14900 14910 14920 14930 14940
GTGGGAACAA ACGGCGGATT GACCGTAATG GGATAGGTGA CGTTGGTGTA GATGGGCGCA

14950 14960 14970 14980 14990 15000
TCGTAACCGT GCATCTGCCA GTTTGAGGGG ACGACGACAG TATCGGCCTC AGGAAGATCG

15010 15020 15030 15040 15050 15060
CACTCCAGCC AGCTTTCCGG CACCGCTTCT GGTGCCGGAA ACCAGGCAAA GCGCCATTCG

15070 15080 15090 15100 15110 15120
CCATTCAGGC TGCGCAACTG TTGGGAAGGG CGATCGGTGC GGGCCTCTTC GCTATTACGC

15130 15140 15150 15160 15170 15180
CAGCTGGCGA AAGGGGGATG TGCTGCAAGG CGATTAAGTT GGGTAACGCC AGGGTTTTCC

15190 15200 15210 15220 15230 15240
CAGTCACGAC GTTGTAAAAC GACTTAATCC GTCGAGGGGC TGCCTCGAAG CAGACGACCT

15250 15260 15270 15280 15290 15300
TCCGTTGTGC AGCCAGCGGC GCCTGCGCCG GTGCCCACAA TCGTGCGCGA ACAAACTAAA

15310 15320 15330 15340 15350 15360
CCAGAACAAA TTATACCGGC GGCACCGCCG CCACCACCTT CTCCCGTGCC TAACATTCCA

15370 15380 15390 15400 15410 15420
GCGCCTCCAC CACCACCACC ACCATCGATG TCTGAATTGC CGCCCGCTCC ACCAATGCCG

15430 15440 15450 15460 15470 15480
ACGGAACCTC AACCCGCTGC ACCTTTAGAC GACAGACAAC AATTGTTGGA AGCTATTAGA

15490 15500 15510 15520 15530 15540
AACGAAAAAA ATCGCACTCG TCTCAGACCG GTCAAACCAA AAACGGCGCC CGAAACCAGT

15550 15560 15570 15580 15590 15600
ACAATAGTTG AGGTGCCGAC TGTGTTGCCT AAAGAGACAT TTGAGCCTAA ACCGCCGTCT

DNASIS
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15610 15620 15630 15640 15650 15660
GCATCACCGC CACCACCTCC GCCTCCGCCT CCGCCGCCAG CCCCGCCTGC GCCTCCACCG

15670 15680 15690 15700 15710 15720
ATGGTAGATT TATCATCAGC TCCACCACCG CCGCCATTAG TAGATTTGCC GTCTGAAATG

15730 15740 15750 15760 15770 15780
TTACCACCGC CTGCACCATC GCTTTCTAAC GTGTTGTCTG AATTAAAATC GGGCACAGTT

15790 15800 15810 15820 15830 15840
AGATTGAAAC CCGCCCAAAA ACGCCCGCAA TCAGAAATAA TTCCAAAAAG CTCAACTACA

15850 15860 15870 15880 15890 15900
AATTTGATCG CGGACGTGTT AGCCGACACA ATTAATAGGC GTCGTGTGGC TATGGCAAAA

15910 15920 15930 15940 15950 15960
TCGTCTTCGG AAGCAACTTC TAACGACGAG GGTTGGGACG ACGACGATAA TCGGCCTAAT

15970 15980 15990 16000 16010 16020
AGCTAACA CGCCCGATGT TAAATATGTC CAAGCTACTA GTGGTACCGC TTGGCAGAAC

16030 16040 16050 16060 16070 16080
ATATCCATCG CGTCCGCCAT CTCCAGCAGC CGCACGCGGC GCATCTCGGG CAGCGTTGGG

16090 16100 16110 16120 16130 16140
TCCTGGCCAC GGGTGCGCAT GATCGTGCTC CTGTCGTTGA GGACCCGGCT AGGCTGGCGG

16150 16160 16170 16180 16190 16200
GGTTGCCTTA CTGGTTAGCA GAATGAATCA CCGATACGCG AGCGAACGTG AAGCGACTGC

16210 16220 16230 16240 16250 16260
TGCTGCAAAA CGTCTGCGAC CTGAGCAACA ACATGAATGG TCTTCGGTTT CCGTGTTTCG

16270 16280 16290 16300 16310 16320
TAAAGTCTGG AAACGCGGAA GTCAGCGCCC TGCACCATTA TGTTCCGGAT CTGCATCGCA

16330 16340 16350 16360 16370 16380
GATGCTGCT GGCTACCCTG TGGAACACCT ACATCTGTAT TAACGAAGCG CTGGCATTGA

16390 16400 16410 16420 16430 16440
CCCTGAGTGA TTTTTCTCTG GTCCCGCCGC ATCCATACCG CCAGTTGTTT ACCCTCACAA

16450 16460 16470 16480 16490 16500
CGTTCCAGTA ACCGGGCATG TTCATCATCA GTAACCCGTA TCGTGAGCAT CCTCTCTCGT

16510 16520 16530 16540 16550 16560
TTCATCGGTA TCATTACCCC CATGAACAGA AATCCCCCTT ACACGGAGGC ATCAGTGACC

16570 16580 16590 16600 16610 16620
AAACAGGAAA AAACCGCCCT TAACATGGCC CGCTTTATCA GAAGCCAGAC ATTAACGCTT

16630 16640 16650 16660 16670 16680
CTGGAGAAAC TCAACGAGCT GGACGCGGAT GAACAGGCAG ACATCTGTGA ATCGCTTCAC

16690 16700 16710 16720 16730 16740
GACCACGCTG ATGAGCTTTA CCGCAGCTGC CTCGCGCGTT TCGGTGATGA CGGTGAAAAC

16750 16760 16770 16780 16790 16800
CTCTGACACA TGCAGCTCCC GGAGACGGTC ACAGCTTGTC TGTAAGCGGA TGCCGGGAGC

16810 16820 16830 16840 16850 16860
AGACAAGCCC GTCAGGGCGC GTCAGCGGGT GTTGGCGGGT GTCGGGGCGC AGCCATGACC

16870 16880 16890 16900 16910 16920
CAGTCACGTA GCGATAGCGG AGTGTATACT GGCTTAACTA TGCGGCATCA GAGCAGATTG

DNASIS
Molly Lark

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     16930      16940      16950      16960      16970      16980
TACTGAGAGT GCACCATATG CGGTGTGAAA TACCGCACAG ATGCGTAAGG AGAAAATACC

     16990      17000      17010      17020      17030      17040
GCATCAGGCG CTCTTCCGCT TCCTCGCTCA CTGACTCGCT GCGCTCGGTC GTTCGGCTGC

     17050      17060      17070      17080      17090      17100
GGCGAGCGGT ATCAGCTCAC TCAAAGGCGG TAATACGGTT ATCCACAGAA TCAGGGGATA

     17110      17120      17130      17140      17150      17160
ACGCAGGAAA GAACATGTGA GCAAAAGGCC AGCAAAAGGC CAGGAACCGT AAAAAGGCCG

     17170      17180      17190      17200      17210      17220
CGTTGCTGGC GTTTTTCCAT AGGCTCCGCC CCCCTGACGA GCATCACAAA AATCGACGCT

     17230      17240      17250      17260      17270      17280
CAAGTCAGAG GTGGCGAAAC CCGACAGGAC TATAAAGATA CCAGGCGTTT CCCCCTGGAA

     17290      17300      17310      17320      17330      17340
GCTCCCTCGT GCGCTCTCCT GTTCCGACCC TGCCGCTTAC CGGATACCTG TCCGCCTTTC

     17350      17360      17370      17380      17390      17400
TCCCTTCGGG AAGCGTGGCG CTTTCTCATA GCTCACGCTG TAGGTATCTC AGTTCGGTGT

     17410      17420      17430      17440      17450      17460
AGGTCGTTCG CTCCAAGCTG GGCTGTGTGC ACGAACCCCC CGTTCAGCCC GACCGCTGCG

     17470      17480      17490      17500      17510      17520
CCTTATCCGG TAACTATCGT CTTGAGTCCA ACCCGGTAAG ACACGACTTA TCGCCACTGG

     17530      17540      17550      17560      17570      17580
CAGCAGCCAC TGGTAACAGG ATTAGCAGAG CGAGGTATGT AGGCGGTGCT ACAGAGTTCT

     17590      17600      17610      17620      17630      17640
TGAAGTGGTG GCCTAACTAC GGCTACACTA GAAGGACAGT ATTTGGTATC TGCGCTCTGC

     17650      17660      17670      17680      17690      17700
TGAAGCCAGT TACCTTCGGA AAAAGAGTTG GTAGCTCTTG ATCCGGCAAA CAAACCACCG

     17710      17720      17730      17740      17750      17760
CTGGTAGCGG TGGTTTTTTT GTTTGCAAGC AGCAGATTAC GCGCAGAAAA AAAGGATCTC

     17770      17780      17790      17800      17810      17820
AAGAAGATCC TTTGATCTTT TCTACGGGGT CTGACGCTCA GTGGAACGAA AACTCACGTT

     17830      17840      17850      17860      17870      17880
AAGGGATTTT GGTCATGAGA TTATCAAAAA GGATCTTCAC CTAGATCCTT TTAAATTAAA

     17890      17900      17910      17920      17930      17940
AATGAAGTTT TAAATCAATC TAAAGTATAT ATGAGTAAAC TTGGTCTGAC AGTTACCAAT

     17950      17960      17970      17980      17990      18000
GCTTAATCAG TGAGGCACCT ATCTCAGCGA TCTGTCTATT TCGTTCATCC ATAGTTGCCT

     18010      18020      18030      18040      18050      18060
GACTCCCCGT CGTGTAGATA ACTACGATAC GGGAGGGCTT ACCATCTGGC CCCAGTGCTG

     18070      18080      18090      18100      18110      18120
CAATGATACC GCGAGACCCA CGCTCACCGG CTCCAGATTT ATCAGCAATA AACCAGCCAG

     18130      18140      18150      18160      18170      18180
CCGGAAGGGC CGAGCGCAGA AGTGGTCCTG CAACTTTATC CGCCTCCATC CAGTCTATTA

     18190      18200      18210      18220      18230      18240
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DNASIS
Molly Lark

ATTGTTGCCG GGAAGCTAGA GTAAGTAGTT CGCCAGTTAA TAGTTTGCGC AACGTTGTTG

18250 18260 18270 18280 18290 18300
CCATTGCTGC AGGCATCGTG GTGTCACGCT CGTCGTTTGG TATGGCTTCA TTCAGCTCCG

18310 18320 18330 18340 18350 18360
GTTCCCAACG ATCAAGGCGA GTTACATGAT CCCCCATGTT GTGCAAAAAA GCGGTTAGCT

18370 18380 18390 18400 18410 18420
CCTTCGGTCC TCCGATCGTT GTCAGAAGTA AGTTGGCCGC AGTGTTATCA CTCATGGTTA

18430 18440 18450 18460 18470 18480
TGGCAGCACT GCATAATTCT CTTACTGTCA TGCCATCCGT AAGATGCTTT TCTGTGACTG

18490 18500 18510 18520 18530 18540
GTGAGTACTC AACCAAGTCA TTCTGAGAAT AGTGTATGCG GCGACCGAGT TGCTCTTGCC

18550 18560 18570 18580 18590 18600
CGGCGTCAAC ACGGGATAAT ACCGCGCCAC ATAGCAGAAC TTTAAAAGTG CTCATCATTG

18610 18620 18630 18640 18650 18660
GAAAACGTTC TTCGGGGCGA AAACTCTCAA GGATCTTACC GCTGTTGAGA TCCAGTTCGA

18670 18680 18690 18700 18710 18720
TGTAACCCAC TCGTGCACCC AACTGATCTT CAGCATCTTT TACTTTCACC AGCGTTTCTG

18730 18740 18750 18760 18770 18780
GGTGAGCAAA AACAGGAAGG CAAAATGCCG CAAAAAAGGG AATAAGGGCG ACACGGAAAT

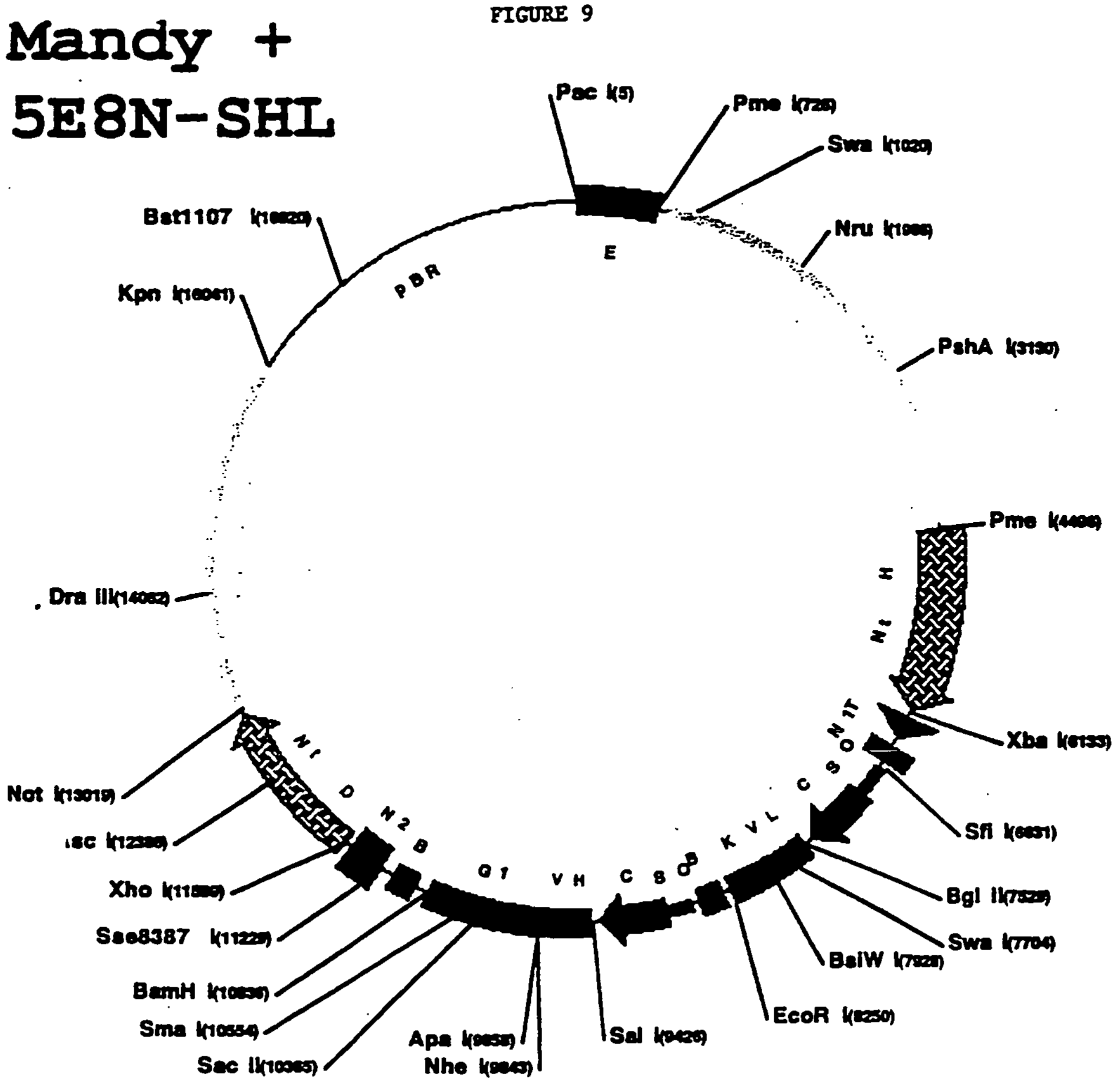
18790 18800 18810 18820 18830 18840
GTTGAATACT CATACTCTTC CTTTTTCAAT ATTATTGAAG CATTTATCAG GGTTATTGTC

18850 18860 18870 18880 18890 18900
TCATGAGCGG ATACATATTT GAATGTATTT AGAAAAATAA ACAAATAGGG GTTCCGCGCA

18910 18920 18930 18940 18950 18960
CATTTCCCCG AAAAGTGCCA CCTGACGTCT AAGAAACCAT TATTATCATG ACATTAACCT

18970 18980 18990 19000 19010 19020
ATAAAAATAG GCGTATCACG AGGCCCTTTC GTCTTCAAGA A......... ....

FIGURE 9



Nt D = Inactive Dihydrofolate reductase   SO = SV40 Origin of replication
E = CMV and SV40 enhancers
Nt H = Inactive Samonella Histidinol Dehydrogenase
T = Herpes Simplex thymidine kinas promoter and polyoma enhancer
C = Cytomegaloviurs promoter/enhancer   B = Bovine growth hormone polyadenylation
N1 = Neomycin phosphotransferase exon 1   N2 = Neomycin phosphotransferase exon 2
K = Human kappa constant   G1 = Human Gamma 1 constant
VL = Variable light chain anti-CD23 primate 5E8 and leader
VH = Variable heavy chain anti-CD23 primate 5E8N- and leader

Mandy cut XbaI Xho I and ligated to Xba I Xho I fragment from XKG1+CD23 5E8N-SHL

Map by Mitchell Reff   Constructed by Karen McLachlan   08/26/97   19,035 bp
Noncutters = AflII, AvrII, HindIII, I-PpoI, I-SceI, PmlI, RsrII, SgfI, SrfI

FIGURE 10

DNASIS
Mandy + 5E8N-SHL

10	20	30	40	50	60
TTAATTAAGG	GGCGGAGAAT	GGGCGGAACT	GGGCGGAGTT	AGGGGCGGGA	TGGGCGGAGT
70	80	90	100	110	120
TAGGGGCGGG	ACTATGGTTG	CTGACTAATT	GAGATGCATG	CTTTGCATAC	TTCTGCCTGC
130	140	150	160	170	180
TGGGGAGCCT	GGGGACTTTC	CACACCTGGT	TGCTGACTAA	TTGAGATGCA	TGCTTTGCAT
190	200	210	220	230	240
ACTTCTGCCT	GCTGGGGAGC	CTGGGGACTT	TCCACACCCT	AACTGACACA	CATTCCACAG
250	260	270	280	290	300
AATTAATTCC	CCTAGTTATT	AATAGTAATC	AATTACGGGG	TCATTAGTTC	ATAGCCCATA
310	320	330	340	350	360
TATGGAGTTC	CGCGTTACAT	AACTTACGGT	AAATGGCCCG	CCTGGCTGAC	CGCCCAACGA
370	380	390	400	410	420
CCCCCGCCCA	TTGACGTCAA	TAATGACGTA	TGTTCCCATA	GTAACGCCAA	TAGGGACTTT
430	440	450	460	470	480
CCATTGACGT	CAATGGGTGG	AGTATTTACG	GTAAACTGCC	CACTTGGCAG	TACATCAAGT
490	500	510	520	530	540
GTATCATATG	CCAAGTACGC	CCCCTATTGA	CGTCAATGAC	GGTAAATGGC	CCGCCTGGCA
550	560	570	580	590	600
TTATGCCCAG	TACATGACCT	TATGGGACTT	TCCTACTTGG	CAGTACATCT	ACGTATTAGT
610	620	630	640	650	660
CATCGCTATT	ACCATGGTGA	TGCGGTTTTG	GCAGTACATC	AATGGGCGTG	GATAGCGGTT
670	680	690	700	710	720
TGACTCACGG	GGATTTCCAA	GTCTCCACCC	CATTGACGTC	AATGGGAGTT	TGTTTTGAAG
730	740	750	760	770	780
[illegible]GTTTAAAC	AGCTTGGCCG	GCCAGCTTTA	TTTAACGTGT	TTACGTCGAG	TCAATTGTAC
790	800	810	820	830	840
ACTAACGACA	GTGATGAAAG	AAATACAAAA	GCGCATAATA	TTTTGAACGA	CGTCGAACCT
850	860	870	880	890	900
TTATTACAAA	ACAAAACACA	AACGAATATC	GACAAAGCTA	GATTGCTGCT	ACAAGATTTG
910	920	930	940	950	960
GCAAGTTTTG	TGGCGTTGAG	CGAAAATCCA	TTAGATAGTC	CAGCCATCGG	TTCGGAAAAA
970	980	990	1000	1010	1020
CAACCCTTGT	TTGAAACTAA	TCGAAACCTA	TTTTACAAAT	CTATTGAGGA	TTTAATATTT
1030	1040	1050	1060	1070	1080
AAATTCAGAT	ATAAAGACGC	TGAAAATCAT	TTGATTTTCG	CTCTAACATA	CCACCCTAAA
1090	1100	1110	1120	1130	1140
GATTATAAAT	TTAATGAATT	ATTAAAATAC	ATCAGCAACT	ATATATTGAT	AGACATTTCC
1150	1160	1170	1180	1190	1200
AGTTTGTGAT	ATTAGTTTGT	GCGTCTCATT	ACAATGGCTG	TTATTTTTAA	CAACAAACAA
1210	1220	1230	1240	1250	1260
CTGCTCGCAG	ACAATAGTAT	AGAAAAGGGA	GGTGAACTGT	TTTTGTTTAA	CGGTTCGTAC
1270	1280	1290	1300	1310	1320
AACATTTTGG	AAAGTTATGT	TAATCCGGTG	CTGCTAAAAA	ATGGTGTAAT	TGAACTAGAA

DNASIS
Mandy + 5E8N-SHL

1330 1340 1350 1360 1370 1380
GAAGCTGCGT ACTATGCCGG CAACATATTG TACAAAACCG ACGATCCCAA ATTCATTGAT

1390 1400 1410 1420 1430 1440
TATATAAATT TAATAATTAA AGCAACACAC TCCGAAGAAC TACCAGAAAA TAGCACTGTT

1450 1460 1470 1480 1490 1500
GTAAATTACA GAAAAACTAT GCGCAGCGGT ACTATACACC CCATTAAAAA AGACATATAT

1510 1520 1530 1540 1550 1560
ATTTATGACA ACAAAAAATT TACTCTATAC GATAGATACA TATATGGATA CGATAATAAC

1570 1580 1590 1600 1610 1620
TATGTTAATT TTTATGAGGA GAAAAATGAA AAAGAGAAGG AATACGAAGA AGAAGACGAC

1630 1640 1650 1660 1670 1680
AAGGCGTCTA GTTTATGTGA AAATAAAATT ATATTGTCGC AAATTAACTG TGAATCATTT

1690 1700 1710 1720 1730 1740
GAAAATGATT TTAAATATTA CCTCAGCGAT TATAACTACG CGTTTTCAAT TATAGATAAT

1750 1760 1770 1780 1790 1800
ACTACAAATG TTCTTGTTGC GTTTGGTTTG TATCGTTAAT AAAAAACAAA TTTGACATTT

1810 1820 1830 1840 1850 1860
ATAATTGTTT TATTATTCAA TAATTACAAA TAGGATTGAG ACCCTTGCAG TTGCCAGCAA

1870 1880 1890 1900 1910 1920
ACGGACAGAG CTTGTCGAGG AGAGTTGTTG ATTCATTGTT TGCCTCCCTG CTGCGGTTTT

1930 1940 1950 1960 1970 1980
TCACCGAAGT TCATGCCAGT CCAGCGTTTT TGCAGCAGAA AAGCCGCCGA CTTCGGTTTG

1990 2000 2010 2020 2030 2040
CGGTCGCGAG TGAAGATCCC TTTCTTGTTA CCGCCAACGC GCAATATGCC TTGCGAGGTC

2050 2060 2070 2080 2090 2100
GCAAAATCGG CGAAATTCCA TACCTGTTCA CCGACGACGG CGCTGACGCG ATCAAAGACG

2110 2120 2130 2140 2150 2160
CGGTGATACA TATCCAGCCA TGCACACTGA TACTCTTCAC TCCACATGTC GGTGTACATT

2170 2180 2190 2200 2210 2220
GAGTGCAGCC CGGCTAACGT ATCCACGCCG TATTCGGTGA TGATAATCGG CTGATGCAGT

2230 2240 2250 2260 2270 2280
TTCTCCTGCC AGGCCAGAAG TTCTTTTTCC AGTACCTTCT CTGCCGTTTC CAAATCGCCG

2290 2300 2310 2320 2330 2340
CTTTGGACAT ACCATCCGTA ATAACGGTTC AGGCACAGCA CATCAAAGAG ATCGCTGATG

2350 2360 2370 2380 2390 2400
GTATCGGTGT GAGCGTCGCA GAACATTACA TTGACGCAGG TGATCGGACG CGTCGGGTCG

2410 2420 2430 2440 2450 2460
AGTTTACGCG TTGCTTCCGC CAGTGGCGCG AAATATTCCC GTGCACCTTG CGGACGGGTA

2470 2480 2490 2500 2510 2520
TCCGGTTCGT TGGCAATACT CCACATCACC ACGCTTGGGT GGTTTTTGTC ACGCGCTATC

2530 2540 2550 2560 2570 2580
AGCTCTTTAA TCGCCTGTAA GTGCGCTTGC TGAGTTTCCC CGTTGACTGC CTCTTCGCTG

2590 2600 2610 2620 2630 2640

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TACAGTTCTT TCGGCTTGTT GCCCGCTTCG AAACCAATGC CTAAAGAGAG GTTAAAGCCG

      2650       2660       2670       2680       2690       2700
ACAGCAGCAG TTTCATCAAT CACCACGATG CCATGTTCAT CTGCCCAGTC GAGCATCTCT

      2710       2720       2730       2740       2750       2760
TCAGCGTAAG GGTAATGCGA GGTACGGTAG GAGTTGGCCC CAATCCAGTC CATTAATGCG

      2770       2780       2790       2800       2810       2820
TGGTCGTGCA CCATCAGCAC GTTATCGAAT CCTTTGCCAC GCAAGTCCGC ATCTTCATGA

      2830       2840       2850       2860       2870       2880
CGACCAAAGC CAGTAAAGTA GAACGGTTTG TGGTTAATCA GGAACTGTTC GCCCTTCACT

      2890       2900       2910       2920       2930       2940
GCCACTGACC GGATGCCGAC GCGAAGCGGG TAGATATCAC ACTCTGTCTG GCTTTTGGCT

      2950       2960       2970       2980       2990       3000
 TGACGCACA GTTCATAGAG ATAACCTTCA CCCGGTTGCC AGAGGTGCGG ATTCACCACT

      3010       3020       3030       3040       3050       3060
TGCAAAGTCC CGCTAGTGCC TTGTCCAGTT GCAACCACCT GTTGATCCGC ATCACGCAGT

      3070       3080       3090       3100       3110       3120
TCAACGCTGA CATCACCATT GGCCACCACC TGCCAGTCAA CAGACGCGTG GTTACAGTCT

      3130       3140       3150       3160       3170       3180
TGCGCGACAT GCGTCACCAC GGTGATATCG TCCACCCAGG TGTTCGGCGT GGTGTAGAGC

      3190       3200       3210       3220       3230       3240
ATTACGCTGC GATGGATTCC GGCATAGTTA AAGAAATCAT GGAAGTAAGA CTGCTTTTTC

      3250       3260       3270       3280       3290       3300
TTGCCGTTTT CGTCGGTAAT CACCATTCCC GGCGGGATAG TCTGCCAGTT CAGTTCGTTG

      3310       3320       3330       3340       3350       3360
 TCACACAAA CGGTGATACC CCTCGACGGA TTAAAGACTT CAAGCGGTCA ACTATGAAGA

      3370       3380       3390       3400       3410       3420
AGTGTTCGTC TTCGTCCCAG TAAGCTATGT CTCCAGAATG TAGCCATCCA TCCTTGTCAA

      3430       3440       3450       3460       3470       3480
TCAAGGCGTT GGTCGCTTCC GGATTGTTTA CATAACCGGA CATAATCATA GGTCCTCTGA

      3490       3500       3510       3520       3530       3540
CACATAATTC GCCTCTCTGA TTAACGCCCA GCGTTTTCCC GGTATCCAGA TCCACAACCT

      3550       3560       3570       3580       3590       3600
TCGCTTCAAA AAATGGAACA ACTTTACCGA CCGCGCCCGG TTTATCATCC CCCTCGGGTG

      3610       3620       3630       3640       3650       3660
TAATCAGAAT AGCTGATGTA GTCTCAGTGA GCCCATATCC TTGTCGTATC CCTGGAAGAT

      3670       3680       3690       3700       3710       3720
GGAAGCGTTT TGCAACCGCT TCCCCGACTT CTTTCGAAAG AGGTGCGCCC CCAGAAGCAA

      3730       3740       3750       3760       3770       3780
TTTCGTGTAA ATTAGATAAA TCGTATTTGT CAATCAGAGT GCTTTTGGCG AAGAATGAAA

      3790       3800       3810       3820       3830       3840
ATAGGGTTGG TACTAGCAAC GCACTTTGAA TTTTGTAATC CTGAAGGGAT CGTAAAAACA

      3850       3860       3870       3880       3890       3900
GCTCTTCTTC AAATCTATAC ATTAAGACGA CTCGAAATCC ACATATCAAA TATCCGAGTG
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      3910       3920       3930       3940       3950       3960
TAGTAAACAT TCCAAAACCG TGATGGAATG GAACAACACT TAAAATCGCA GTATCCGGAA

      3970       3980       3990       4000       4010       4020
TGATTTGATT GCCAAAAATA GGATCTCTGG CATGCGAGAA TCTGACGCAG GCAGTTCTAT

      4030       4040       4050       4060       4070       4080
GCGGAAGGGC CACACCCTTA GGTAACCCAG TAGATCCAGA GGAATTGTTT TGTCACGATC

      4090       4100       4110       4120       4130       4140
AAAGGACTCT GGTACAAAAT CGTATTCATT AAAACCGGGA GGTAGATGAG ATGTGACGAA

      4150       4160       4170       4180       4190       4200
CGTGTACATC GACTGAAATC CCTGGTAATC CGTTTTAGAA TCCATGATAA TAATTTTCTG

      4210       4220       4230       4240       4250       4260
GATTATTGGT AATTTTTTTT GCACGTTCAA AATTTTTTGC AACCCCTTTT TGGAAACAAA

      4270       4280       4290       4300       4310       4320
 .CTACGGTA GGCTGCGAAA TGTTCATACT GTTGAGCAAT TCACGTTCAT TATAAATGTC

      4330       4340       4350       4360       4370       4380
GTTCGCGGGC GCAACTGCAA CTCCGATAAA TAACGCGCCC AACACCGGCA TAAAGAATTG

      4390       4400       4410       4420       4430       4440
AAGAGAGTTT TCACTGCATA CGACGATTCT GTGATTTGTA TTCAGCCCAT ATCGTTTCAT

      4450       4460       4470       4480       4490       4500
AGCTTCTGCC AACCGAACGG ACATTTCGAA GTATTCCGCG TACAGCCCGG CCGTTTAAAC

      4510       4520       4530       4540       4550       4560
GGCCGGGCTT CAATACCCTG ATTGACTGGA ACAGCTGTAG CCCTGAACAG CAGCGTGCGC

      4570       4580       4590       4600       4610       4620
TGCTGACGCG TCCGGCGATT TCCGCCTCTG ACAGTATTAC CCGGACGGTC AGCGATATTC

      4630       4640       4650       4660       4670       4680
 .GATAATGT AAAAACGCGC GGTGACGATG CCCTGCGTGA ATACAGCGCT AAATTTGATA

      4690       4700       4710       4720       4730       4740
AAACAGAAGT GACAGCGCTA CGCGTCACCC CTGAAGAGAT CGCCGCCGCC GGCGCGCGTC

      4750       4760       4770       4780       4790       4800
TGAGCGACGA ATTAAAACAG GCGATGACCG CTGCCGTCAA AAATATTGAA ACGTTCCATT

      4810       4820       4830       4840       4850       4860
CCGCGCAGAC GCTACCGCCT GTAGATGTGG AAACCCAGCC AGGCGTGCGT TGCCAGCAGG

      4870       4880       4890       4900       4910       4920
TTACGCGTCC CGTCTCGTCT GTCGGTCTGT ATATTCCCGG CGGCTCGGCT CCGCTCTTCT

      4930       4940       4950       4960       4970       4980
CAACGGTGCT GATGCTGGCG ACGCCGGCGC GCATTGCGGG ATGCCAGAAG GTGGTTCTGT

      4990       5000       5010       5020       5030       5040
GCTCGCCGCC GCCCATCGCT GATGAAATCC TCTATGCGGC GCAACTGTGT GGCGTGCAGG

      5050       5060       5070       5080       5090       5100
AAATCTTTAA CGTCGGCGGC GCGCAGGCGA TTGCCGCTCT GGCCTTCGGC AGCGAGTCCG

      5110       5120       5130       5140       5150       5160
TACCGAAAGT GGATAAAATT TTTGGCCCCG GCAACGCCTT TGTAACCGAA GCCAAACGTC

      5170       5180       5190       5200       5210       5220
AGGTCAGCCA GCGTCTCGAC GGCGCGGCTA TCGATATGCC AGCCGGGCCG TCTGAAGTAC
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5230 5240 5250 5260 5270 5280
TGGTGATCGC AGACAGCGGC GCAACACCGG ATTTCGTCGC TTCTGACCTG CTCTCCCAGG

5290 5300 5310 5320 5330 5340
CTGAGCACGG CCCGGATTCC CAGGTGATCC TGCTGACGCC TGATGCTGAC ATTGCCCGCA

5350 5360 5370 5380 5390 5400
AGGTGGCGGA GGCGGTAGAA CGTCAACTGG CGGAACTGCC GCGCGCGGAC ACCGCCCGGC

5410 5420 5430 5440 5450 5460
AGGCCCTGAG CGCCAGTCGT CTGATTGTGA CCAAAGATTT AGCGCAGTGC GTCGCCATCT

5470 5480 5490 5500 5510 5520
CTAATCAGTA TGGGCCGGAA CACTTAATCA TCCAGACGCG CAATGCGCGC GATTTGGTGG

5530 5540 5550 5560 5570 5580
ATGCGATTAC CAGCGCAGGC TCGGTATTTC TCGGCGACTG GTCGCCGGAA TCCGCCGGTG

5590 5600 5610 5620 5630 5640
ATTACGCTTC CGGAACCAAC CATGTTTTAC CGACCTATGG CTATACTGCT ACCTGTTCCA

5650 5660 5670 5680 5690 5700
GCCTTGGGTT AGCGGATTTC CAGAAACGGA TGACCGTTCA GGAACTGTCG AAAGCGGGCT

5710 5720 5730 5740 5750 5760
TTTCCGCTCT GGCATCAACC ATTGAAACAT TGGCGGCGGC AGAACGTCTG ACCGCCCATA

5770 5780 5790 5800 5810 5820
AAAATGCCGT GACCCTGCGC GTAAACGCCC TCAAGGAGCA AGCATGAGCA CTGAAAACAC

5830 5840 5850 5860 5870 5880
TCTCAGCGTC GCTGACTTAG CCCGTGAAAA TGTCCGCAAC CTGGAGATCC AGACATGGAT

5890 5900 5910 5920 5930 5940
AAGATACATT GATGAGTTTG GACAAACCAC AACTAGAATG CAGTGAAAAA AATGCTTTAT

5950 5960 5970 5980 5990 6000
TTGTGAAATT TGTGATGCTA TTGCTTTATT TGTAACCATT ATAAGCTGCA ATAAACAAGT

6010 6020 6030 6040 6050 6060
TAACAACAAC AATTGCATTC ATTTTATGTT TCAGGTTCAG GGGGAGGTGT GGGAGGTTTT

6070 6080 6090 6100 6110 6120
TTAAAGCAAG TAAAACCTCT ACAAATGTGG TATGGCTGAT TATGATCTCT AGGGCCGGCC

6130 6140 6150 6160 6170 6180
CTCGACGGCG CGTCTAGAGC AGTGTGGTTT TCAAGAGGAA GCAAAAAGCC TCTCCACCCA

6190 6200 6210 6220 6230 6240
GGCCTGGAAT GTTTCCACCC AATGTCGAGC AGTGTGGTTT TGCAAGAGGA AGCAAAAAGC

6250 6260 6270 6280 6290 6300
CTCTCCACCC AGGCCTGGAA TGTTTCCACC CAATGTCGAG CAAACCCCGC CCAGCGTCTT

6310 6320 6330 6340 6350 6360
GTCATTGGCG AATTGGAACA CGCATATGCA GTCGGGGCGG CGCGGTCCCA GGTCCACTTC

6370 6380 6390 6400 6410 6420
GCATATTAAG GTGGCGCGTG TGGCCTCGAA CACCGAGCGA CCCTGCAGCC AATATGGGAT

6430 6440 6450 6460 6470 6480
CGGCCATTGA ACAAGATGGA TTGCACGCAG GTTCTCCGGC CGCTTGGGTG GAGAGGCTAT

6490 6500 6510 6520 6530 6540

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TCGGCTATGA CTGGGCACAA CAGACAATCG GCTGCTCTGA TGCCGCCGTG TTCCGGCTGT

6550 6560 6570 6580 6590 6600
CAGCGCAGGG GCGCCCGGTT CTTTTTGTCA AGACCGACCT GTCCGGTGCC CTGAATGAAC

6610 6620 6630 6640 6650 6660
TGCAGGTAAG TGCGGCCGTC GATGGCCGAG GCGGCCTCGG CCTCTGCATA AATAAAAAAA

6670 6680 6690 6700 6710 6720
ATTAGTCAGC CATGCATGGG GCGGAGAATG GGCGGAACTG GGCGGAGTTA GGGGCGGGAT

6730 6740 6750 6760 6770 6780
GGGCGGAGTT AGGGGCGGGA CTATGGTTGC TGACTAATTG AGATGCATGC TTTGCATACT

6790 6800 6810 6820 6830 6840
TCTGCCTGCT GGGGAGCCTG GGGACTTTCC ACACCTGGTT GCTGACTAAT TGAGATGCAT

6850 6860 6870 6880 6890 6900
GCTTTGCATA CTTCTGCCTG CTGGGGAGCC TGGGGACTTT CCACACCCTA ACTGACACAC

6910 6920 6930 6940 6950 6960
ATTCCACAGA ATTAATTCCC CTAGTTATTA ATAGTAATCA ATTACGGGGT CATTAGTTCA

6970 6980 6990 7000 7010 7020
TAGCCCATAT ATGGAGTTCC GCGTTACATA ACTTACGGTA AATGGCCCGC CTGGCTGACC

7030 7040 7050 7060 7070 7080
GCCCAACGAC CCCCGCCCAT TGACGTCAAT AATGACGTAT GTTCCCATAG TAACGCCAAT

7090 7100 7110 7120 7130 7140
AGGGACTTTC CATTGACGTC AATGGGTGGA GTATTTACGG TAAACTGCCC ACTTGGCAGT

7150 7160 7170 7180 7190 7200
ACATCAAGTG TATCATATGC CAAGTACGCC CCCTATTGAC GTCAATGACG GTAAATGGCC

7210 7220 7230 7240 7250 7260
GGCCTGGCAT TATGCCCAGT ACATGACCTT ATGGGACTTT CCTACTTGGC AGTACATCTA

7270 7280 7290 7300 7310 7320
CGTATTAGTC ATCGCTATTA CCATGGTGAT GCGGTTTTGG CAGTACATCA ATGGGCGTGG

7330 7340 7350 7360 7370 7380
ATAGCGGTTT GACTCACGGG GATTTCCAAG TCTCCACCCC ATTGACGTCA ATGGGAGTTT

7390 7400 7410 7420 7430 7440
GTTTTGGCAC CAAAATCAAC GGGACTTTCC AAAATGTCGT AACAACTCCG CCCCATTGAC

7450 7460 7470 7480 7490 7500
GCAAATGGGC GGTAGGCGTG TACGGTGGGA GGTCTATATA AGCAGAGCTG GGTACGTGAA

7510 7520 7530 7540 7550 7560
CCGTCAGATC GCCTGGAGAC GCCATCACAG ATCTCTCACC ATGGACATGA GGGTCCCCGC

7570 7580 7590 7600 7610 7620
TCAGCTCCTG GGGCTCCTTC TGCTCTGGCT CCCAGGTGCC AGATGTGACA TCCAGATGAC

7630 7640 7650 7660 7670 7680
CCAGTCTCCA TCTTCCCTGT CTGCATCTGT AGGGGACAGA GTCACCATCA CTTGCAGGGC

7690 7700 7710 7720 7730 7740
AAGTCAGGAC ATTAGGTATT ATTTAAATTG GTATCAGCAG AAACCAGGAA AAGCTCCTAA

7750 7760 7770 7780 7790 7800
GCTCCTGATC TATGTTGCAT CCAGTTTGCA AAGTGGGGTC CCATCAAGGT TCAGCGGCAG

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      7810       7820       7830       7840       7850       7860
TGGATCTGGG ACAGAGTTCA CTCTCACCGT CAGCAGCCTG CAGCCTGAAG ATTTTGCGAC

      7870       7880       7890       7900       7910       7920
TTATTACTGT CTACAGGTTT ATAGTACCCC TCGGACGTTC GGCCAAGGGA CCAAGGTGGA

      7930       7940       7950       7960       7970       7980
AATCAAACGT ACGGTGGCTG CACCATCTGT CTTCATCTTC CCGCCATCTG ATGAGCAGTT

      7990       8000       8010       8020       8030       8040
GAAATCTGGA ACTGCCTCTG TTGTGTGCCT GCTGAATAAC TTCTATCCCA GAGAGGCCAA

      8050       8060       8070       8080       8090       8100
AGTACAGTGG AAGGTGGATA ACGCCCTCCA ATCGGGTAAC TCCCAGGAGA GTGTCACAGA

      8110       8120       8130       8140       8150       8160
GCAGGACAGC AAGGACAGCA CCTACAGCCT CAGCAGCACC CTGACGCTGA GCAAAGCAGA

      8170       8180       8190       8200       8210       8220
 TACGAGAAA CACAAAGTCT ACGCCTGCGA AGTCACCCAT CAGGGCCTGA GCTCGCCCGT

      8230       8240       8250       8260       8270       8280
CACAAAGAGC TTCAACAGGG GAGAGTGTTG AATTCAGATC CGTTAACGGT TACCAACTAC

      8290       8300       8310       8320       8330       8340
CTAGACTGGA TTCGTGACAA CATGCGGCCG TGATATCTAC GTATGATCAG CCTCGACTGT

      8350       8360       8370       8380       8390       8400
GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT TGACCCTGGA

      8410       8420       8430       8440       8450       8460
AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG

      8470       8480       8490       8500       8510       8520
TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA

      8530       8540       8550       8560       8570       8580
  ACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC

      8590       8600       8610       8620       8630       8640
CAGCTGGGAC TAGTCGCAAT TGGGCGGAGT TAGGGGCGGG ATGGGCGGAG TTAGGGGCGG

      8650       8660       8670       8680       8690       8700
GACTATGGTT GCTGACTAAT TGAGATGCAT GCTTTGCATA CTTCTGCCTG CTGGGGAGCC

      8710       8720       8730       8740       8750       8760
TGGGGACTTT CCACACCTGG TTGCTGACTA ATTGAGATGC ATGCTTTGCA TACTTCTGCC

      8770       8780       8790       8800       8810       8820
TGCTGGGGAG CCTGGGGACT TTCCACACCC TAACTGACAC ACATTCCACA GAATTAATTC

      8830       8840       8850       8860       8870       8880
CCCTAGTTAT TAATAGTAAT CAATTACGGG GTCATTAGTT CATAGCCCAT ATATGGAGTT

      8890       8900       8910       8920       8930       8940
CCGCGTTACA TAACTTACGG TAAATGGCCC GCCTGGCTGA CCGCCCAACG ACCCCCGCCC

      8950       8960       8970       8980       8990       9000
ATTGACGTCA ATAATGACGT ATGTTCCCAT AGTAACGCCA ATAGGGACTT TCCATTGACG

      9010       9020       9030       9040       9050       9060
TCAATGGGTG GAGTATTTAC GGTAAACTGC CCACTTGGCA GTACATCAAG TGTATCATAT

      9070       9080       9090       9100       9110       9120
GCCAAGTACG CCCCCTATTG ACGTCAATGA CGGTAAATGG CCCGCCTGGC ATTATGCCCA
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DNASIS
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      9130       9140       9150       9160       9170       9180
GTACATGACC TTATGGGACT TTCCTACTTG GCAGTACATC TACGTATTAG TCATCGCTGT

      9190       9200       9210       9220       9230       9240
TACCATGGTG ATGCGGTTTT GGCAGTACAT CAATGGGCGT GGATAGCGGT TTGACTCACG

      9250       9260       9270       9280       9290       9300
GGGATTTCCA AGTCTCCACC CCATTGACGT CAATGGGAGT TTGTTTTGGC ACCAAAATCA

      9310       9320       9330       9340       9350       9360
ACGGGACTTT CCAAAATGTC GTAACAACTC CGCCCCATTG ACGCAAATGG GCGGTAGGCG

      9370       9380       9390       9400       9410       9420
TGTACGGTGG GAGGTCTATA TAAGCAGAGC TGGGTACGTG AACCGTCAGA TCGCCTGGAG

      9430       9440       9450       9460       9470       9480
ACGCCGTCGA CATGGGTTGG AGCCTCATCT TGCTCTTCCT TGTCGCTGTT GCTACGCGTG

      9490       9500       9510       9520       9530       9540
.CCTGTCCGA GGTGCAGCTG GTGGAGTCTG GGGGCGGCTT GGCAAAGCCT GGGGGGTCCC

      9550       9560       9570       9580       9590       9600
TGAGACTCTC CTGCGCAGCC TCCGGGTTCA GGTTCACCTT CAATAACTAC TACATGGACT

      9610       9620       9630       9640       9650       9660
GGGTCCGCCA GGCTCCAGGG CAGGGGCTGG AGTGGGTCTC ACGTATTAGT AGTAGTGGTG

      9670       9680       9690       9700       9710       9720
ATCCCACATG GTACGCAGAC TCCGTGAAGG GCAGATTCAC CATCTCCAGA GAGAACGCCA

      9730       9740       9750       9760       9770       9780
AGAACACACT GTTTCTTCAA ATGAACAGCC TGAGAGCTGA GGACACGGCT GTCTATTACT

      9790       9800       9810       9820       9830       9840
GTGCGAGCTT GACTACAGGG TCTGACTCCT GGGGCCAGGG AGTCCTGGTC ACCGTCTCCT

      9850       9860       9870       9880       9890       9900
CAGCTAGCAC CAAGGGCCCA TCGGTCTTCC CCCTGGCACC CTCCTCCAAG AGCACCTCTG

      9910       9920       9930       9940       9950       9960
GGGGCACAGC GGCCCTGGGC TGCCTGGTCA AGGACTACTT CCCCGAACCG GTGACGGTGT

      9970       9980       9990      10000      10010      10020
CGTGGAACTC AGGCGCCCTG ACCAGCGGCG TGCACACCTT CCCGGCTGTC CTACAGTCCT

     10030      10040      10050      10060      10070      10080
CAGGACTCTA CTCCCTCAGC AGCGTGGTGA CCGTGCCCTC CAGCAGCTTG GGCACCCAGA

     10090      10100      10110      10120      10130      10140
CCTACATCTG CAACGTGAAT CACAAGCCCA GCAACACCAA GGTGGACAAG AAAGTTGAGC

     10150      10160      10170      10180      10190      10200
CCAAATCTTG TGACAAAACT CACACATGCC CACCGTGCCC AGCACCTGAA CTCCTGGGGG

     10210      10220      10230      10240      10250      10260
GACCGTCAGT CTTCCTCTTC CCCCCAAAAC CCAAGGACAC CCTCATGATC TCCCGGACCC

     10270      10280      10290      10300      10310      10320
CTGAGGTCAC ATGCGTGGTG GTGGACGTGA GCCACGAAGA CCCTGAGGTC AAGTTCAACT

     10330      10340      10350      10360      10370      10380
GGTACGTGGA CGGCGTGGAG GTGCATAATG CCAAGACAAA GCCGCGGGAG GAGCAGTACA

     10390      10400      10410      10420      10430      10440
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ACAGCACGTA CCGTGTGGTC AGCGTCCTCA CCGTCCTGCA CCAGGACTGG CTGAATGGCA

10450 10460 10470 10480 10490 10500
AGGAGTACAA GTGCAAGGTC TCCAACAAAG CCCTCCCAGC CCCCATCGAG AAAACCATCT

10510 10520 10530 10540 10550 10560
CCAAAGCCAA AGGGCAGCCC CGAGAACCAC AGGTGTACAC CCTGCCCCCA TCCCGGGATG

10570 10580 10590 10600 10610 10620
AGCTGACCAA GAACCAGGTC AGCCTGACCT GCCTGGTCAA AGGCTTCTAT CCCAGCGACA

10630 10640 10650 10660 10670 10680
TCGCCGTGGA GTGGGAGAGC AATGGGCAGC CGGAGAACAA CTACAAGACC ACGCCTCCCG

10690 10700 10710 10720 10730 10740
TGCTGGACTC CGACGGCTCC TTCTTCCTCT ACAGCAAGCT CACCGTGGAC AAGAGCAGGT

10750 10760 10770 10780 10790 10800
GGCAGCAGGG GAACGTCTTC TCATGCTCCG TGATGCATGA GGCTCTGCAC AACCACTACA

10810 10820 10830 10840 10850 10860
CGCAGAAGAG CCTCTCCCTG TCTCCGGGTA AATGAGGATC CGTTAACGGT TACCAACTAC

10870 10880 10890 10900 10910 10920
CTAGACTGGA TTCGTGACAA CATGCGGCCG TGATATCTAC GTATGATCAG CCTCGACTGT

10930 10940 10950 10960 10970 10980
GCCTTCTAGT TGCCAGCCAT CTGTTGTTTG CCCCTCCCCC GTGCCTTCCT TGACCCTGGA

10990 11000 11010 11020 11030 11040
AGGTGCCACT CCCACTGTCC TTTCCTAATA AAATGAGGAA ATTGCATCGC ATTGTCTGAG

11050 11060 11070 11080 11090 11100
TAGGTGTCAT TCTATTCTGG GGGGTGGGGT GGGGCAGGAC AGCAAGGGGG AGGATTGGGA

11110 11120 11130 11140 11150 11160
AGACAATAGC AGGCATGCTG GGGATGCGGT GGGCTCTATG GCTTCTGAGG CGGAAAGAAC

11170 11180 11190 11200 11210 11220
CAGCTGGGGC TCGACAGCAA CGCTAGGTCG AGGCCGCTAC TAACTCTCTC CTCCCTCCTT

11230 11240 11250 11260 11270 11280
TTTCCTGCAG GACGAGGCAG CGCGGCTATC GTGGCTGGCC ACGACGGGCG TTCCTTGCGC

11290 11300 11310 11320 11330 11340
AGCTGTGCTC GACGTTGTCA CTGAAGCGGG AAGGGACTGG CTGCTATTGG GCGAAGTGCC

11350 11360 11370 11380 11390 11400
GGGGCAGGAT CTCCTGTCAT CTCACCTTGC TCCTGCCGAG AAAGTATCCA TCATGGCTGA

11410 11420 11430 11440 11450 11460
TGCAATGCGG CGGCTGCATA CGCTTGATCC GGCTACCTGC CCATTCGACC ACCAAGCGAA

11470 11480 11490 11500 11510 11520
ACATCGCATC GAGCGAGCAC GTACTCGGAT GGAAGCCGGT CTTGTCGATC AGGATGATCT

11530 11540 11550 11560 11570 11580
GGACGAAGAG CATCAGGGGC TCGCGCCAGC CGAACTGTTC GCCAGGTAAG TGAGCTCCAA

11590 11600 11610 11620 11630 11640
TTCAAGCTCT CGAGCTAGGG CGGCCAGCTA GTAGCTTTGC TTCTCAATTT CTTATTTGCA

11650 11660 11670 11680 11690 11700
TAATGAGAAA AAAAGGAAAA TTAATTTTAA CACCAATTCA GTAGTTGATT GAGCAAATGC

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     11710      11720      11730      11740      11750      11760
GTTGCCAAAA AGGATGCTTT AGAGACAGTG TTCTCTGCAC AGATAAGGAC AAACATTATT

     11770      11780      11790      11800      11810      11820
CAGAGGGAGT ACCCAGAGCT GAGACTCCTA AGCCAGTGAG TGGCACAGCA TCCAGGGAGA

     11830      11840      11850      11860      11870      11880
AATATGCTTG TCATCACCGA AGCCTGATTC CGTAGAGCCA CACCCTGGTA AGGGCCAATC

     11890      11900      11910      11920      11930      11940
TGCTCACACA GGATAGAGAG GGCAGGAGCC AGGGCAGAGC ATATAAGGTG AGGTAGGATC

     11950      11960      11970      11980      11990      12000
AGTTGCTCCT CACATTTGCT TCTGACATAG TTGTGTTGGG AGCTTGGATA GCTTGGGGGG

     12010      12020      12030      12040      12050      12060
GGGACAGCTC AGGGCTGCGA TTTCGCGCCA AACTTGACGG CAATCCTAGC GTGAAGGCTG

     12070      12080      12090      12100      12110      12120
 AGGATTTT ATCCCCGCTG CCATCATGGT TCGACCATTG AACTGCATCG TCGCCGTGTC

     12130      12140      12150      12160      12170      12180
CCAAAATATG GGGATTGGCA AGAACGGAGA CCTACCCTGG CCTCCGCTCA GGAACGAGTT

     12190      12200      12210      12220      12230      12240
CAAGTACTTC CAAAGAATGA CCACAACCTC TTCAGTGGAA GGTAAACAGA ATCTGGTGAT

     12250      12260      12270      12280      12290      12300
TATGGGTAGG AAAACCTGGT TCTCCATTCC TGAGAAGAAT CGACCTTTAA AGGACAGAAT

     12310      12320      12330      12340      12350      12360
TAATATAGTT CTCAGTAGAG AACTCAAAGA ACCACCACGA GGAGCTCATT TTCTTGCCAA

     12370      12380      12390      12400      12410      12420
AAGTTTGGAT GATGCCTTAA CGTAGGCGCG CCATTAAGAC TTATTGAACA ACCGGAATTG

     12430      12440      12450      12460      12470      12480
 AAGTAAAG TAGACATGGT TTGGATAGTC GGAGGCAGTT CTGTTTACCA GGAAGCCATG

     12490      12500      12510      12520      12530      12540
AATCAACCAG GCCACCTCAG ACTCTTTGTG ACAAGGATCA TGCAGGAATT TGAAAGTGAC

     12550      12560      12570      12580      12590      12600
ACGTTTTTCC CAGAAATTGA TTTGGGGAAA TATAAACTTC TCCCAGAATA CCCAGGCGTC

     12610      12620      12630      12640      12650      12660
CTCTCTGAGG TCCAGGAGGA AAAAGGCATC AAGTATAAGT TTGAAGTCTA CGAGAAGAAA

     12670      12680      12690      12700      12710      12720
GACTAACAGG AAGATGCTTT CAAGTTCTCT GCTCCCCTCC TAAAGCTATG CATTTTTATA

     12730      12740      12750      12760      12770      12780
AGACCATGGG ACTTTTGCTG GCTTTAGATC AGCCTCGACT GTGCCTTCTA GTTGCCAGCC

     12790      12800      12810      12820      12830      12840
ATCTGTTGTT TGCCCCTCCC CCGTGCCTTC CTTGACCCTG GAAGGTGCCA CTCCCACTGT

     12850      12860      12870      12880      12890      12900
CCTTTCCTAA TAAAATGAGG AAATTGCATC GCATTGTCTG AGTAGGTGTC ATTCTATTCT

     12910      12920      12930      12940      12950      12960
GGGGGGTGGG GTGGGGCAGG ACAGCAAGGG GGAGGATTGG GAAGACAATA GCAGGCATGC

     12970      12980      12990      13000      13010      13020
TGGGGATGCG GTGGGCTCTA TGGCTTCTGA GGCGGAAAGA ACCAGCTGGG GCTCGAAGCG
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DNASIS
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     13030      13040      13050      13060      13070      13080
GCCGCCCATT TCGCTGGTGG TCAGATGCGG GATGGCGTGG GACGCGGCGG GGAGCGTCAC

     13090      13100      13110      13120      13130      13140
ACTGAGGTTT TCCGCCAGAC GCCACTGCTG CCAGGCGCTG ATGTGCCCGG CTTCTGACCA

     13150      13160      13170      13180      13190      13200
TGCGGTCGCG TTCGGTTGCA CTACGCGTAC TGTGAGCCAG AGTTGCCCGG CGCTCTCCGG

     13210      13220      13230      13240      13250      13260
CTGCGGTAGT TCAGGCAGTT CAATCAACTG TTTACCTTGT GGAGCGACAT CCAGAGGCAC

     13270      13280      13290      13300      13310      13320
TTCACCGCTT GCCAGCGGCT TACCATCCAG CGCCACCATC CAGTGCAGGA GCTCGTTATC

     13330      13340      13350      13360      13370      13380
GCTATGACGG AACAGGTATT CGCTGGTCAC TTCGATGGTT TGCCCGGATA AACGGAACTG

     13390      13400      13410      13420      13430      13440
AAAAACTGC TGCTGGTGTT TTGCTTCCGT CAGCGCTGGA TGCGGCGTGC GGTCGGCAAA

     13450      13460      13470      13480      13490      13500
GACCAGACCG TTCATACAGA ACTGGCGATC GTTCGGCGTA TCGCCAAAAT CACCGCCGTA

     13510      13520      13530      13540      13550      13560
AGCCGACCAC GGGTTGCCGT TTTCATCATA TTTAATCAGC GACTGATCCA CCCAGTCCCA

     13570      13580      13590      13600      13610      13620
GACGAAGCCG CCCTGTAAAC GGGGATACTG ACGAAACGCC TGCCAGTATT TAGCGAAACC

     13630      13640      13650      13660      13670      13680
GCCAAGACTG TTACCCATCG CGTGGGCGTA TTCGCAAAGG ATCAGCGGGC GCGTCTCTCC

     13690      13700      13710      13720      13730      13740
AGGTAGCGAA AGCCATTTTT TGATGGACCA TTTCGGCACA GCCGGGAAGG GCTGGTCTTC

     13750      13760      13770      13780      13790      13800
TCCACGCGC GCGTACATCG GGCAAATAAT ATCGGTGGCC GTGGTGTCGG CTCCGCCGCC

     13810      13820      13830      13840      13850      13860
TTCATACTGC ACCGGGCGGG AAGGATCGAC AGATTTGATC CAGCGATACA GCGCGTCGTG

     13870      13880      13890      13900      13910      13920
ATTAGCGCCG TGGCCTGATT CATTCCCCAG CGACCAGATG ATCACACTCG GGTGATTACG

     13930      13940      13950      13960      13970      13980
ATCGCGCTGC ACCATTCGCG TTACGCGTTC GCTCATCGCC GGTAGCCAGC GCGGATCATC

     13990      14000      14010      14020      14030      14040
GGTCAGACGA TTCATTGGCA CCATGCCGTG GGTTTCAATA TTGGCTTCAT CCACCACATA

     14050      14060      14070      14080      14090      14100
CAGGCCGTAG CGGTCGCACA GCGTGTACCA CAGCGGATGG TTCGGATAAT GCGAACAGCG

     14110      14120      14130      14140      14150      14160
CACGGCGTTA AAGTTGTTCT GCTTCATCAG CAGGATATCC TGCACCATCG TCTGCTCATC

     14170      14180      14190      14200      14210      14220
CATGACCTGA CCATGCAGAG GATGATGCTC GTGACGGTTA ACGCCTCGAA TCAGCAACGG

     14230      14240      14250      14260      14270      14280
CTTGCCGTTC AGCAGCAGCA GACCATTTTC AATCCGCACC TCGCGGAAAC CGACATCGCA

     14290      14300      14310      14320      14330      14340
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DNASIS
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GGCTTCTGCT TCAATCAGCG TGCCGTCGGC GGTGTGCAGT TCAACCACCG CACGATAGAG

     14350      14360      14370      14380      14390      14400
ATTCGGGATT TCGGCGCTCC ACAGTTTCGG GTTTTCGACG TTCAGACGTA GTGTGACGCG

     14410      14420      14430      14440      14450      14460
ATCGGCATAA CCACCACGCT CATCGATAAT TTCACCGCCG AAAGGCGCGG TGCCGCTGGC

     14470      14480      14490      14500      14510      14520
GACCTGCGTT TCACCCTGCC ATAAAGAAAC TGTTACCCGT AGGTAGTCAC GCAACTCGCC

     14530      14540      14550      14560      14570      14580
GCACATCTGA ACTTCAGCCT CCAGTACAGC GCGGCTGAAA TCATCATTAA AGCGAGTGGC

     14590      14600      14610      14620      14630      14640
AACATGGAAA TCGCTGATTT GTGTAGTCGG TTTATGCAGC AACGAGACGT CACGGAAAAT

     14650      14660      14670      14680      14690      14700
""CGCTCATC CGCCACATAT CCTGATCTTC CAGATAACTG CCGTCACTCC AGCGCAGCAC

     14710      14720      14730      14740      14750      14760
CATCACCGCG AGGCGGTTTT CTCCGGCGCG TAAAAATGCG CTCAGGTCAA ATTCAGACGG

     14770      14780      14790      14800      14810      14820
CAAACGACTG TCCTGGCCGT AACCGACCCA GCGCCCGTTG CACCACAGAT GAAACGCCGA

     14830      14840      14850      14860      14870      14880
GTTAACGCCA TCAAAAATAA TTCGCGTCTG GCCTTCCTGT AGCCAGCTTT CATCAACATT

     14890      14900      14910      14920      14930      14940
AAATGTGAGC GAGTAACAAC CCGTCGGATT CTCCGTGGGA ACAAACGGCG GATTGACCGT

     14950      14960      14970      14980      14990      15000
AATGGGATAG GTCACGTTGG TGTAGATGGG CGCATCGTAA CCGTGCATCT GCCAGTTTGA

     15010      15020      15030      15040      15050      15060
""GGACGACG ACAGTATCGG CCTCAGGAAG ATCGCACTCC AGCCAGCTTT CCGGCACCGC

     15070      15080      15090      15100      15110      15120
TTCTGGTGCC GGAAACCAGG CAAAGCGCCA TTCGCCATTC AGGCTGCGCA ACTGTTGGGA

     15130      15140      15150      15160      15170      15180
AGGGCGATCG GTGCGGGCCT CTTCGCTATT ACGCCAGCTG GCGAAAGGGG GATGTGCTGC

     15190      15200      15210      15220      15230      15240
AAGGCGATTA AGTTGGGTAA CGCCAGGGTT TTCCCAGTCA CGACGTTGTA AAACGACTTA

     15250      15260      15270      15280      15290      15300
ATCCGTCGAG GGGCTGCCTC GAAGCAGACG ACCTTCCGTT GTGCAGCCAG CGGCGCCTGC

     15310      15320      15330      15340      15350      15360
GCCGGTGCCC ACAATCGTGC GCGAACAAAC TAAACCAGAA CAAATTATAC CGGCGGCACC

     15370      15380      15390      15400      15410      15420
GCCGCCACCA CCTTCTCCCG TGCCTAACAT TCCAGCGCCT CCACCACCAC CACCACCATC

     15430      15440      15450      15460      15470      15480
GATGTCTGAA TTGCCGCCCG CTCCACCAAT GCCGACGGAA CCTCAACCCG CTGCACCTTT

     15490      15500      15510      15520      15530      15540
AGACGACAGA CAACAATTGT TGGAAGCTAT TAGAAACGAA AAAAATCGCA CTCGTCTCAG

     15550      15560      15570      15580      15590      15600
ACCGGTCAAA CCAAAAACGG CGCCCGAAAC CAGTACAATA GTTGAGGTGC CGACTGTGTT
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DNASIS
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     15610      15620      15630      15640      15650      15660
GCCTAAAGAG ACATTTGAGC CTAAACCGCC GTCTGCATCA CCGCCACCAC CTCCGCCTCC

     15670      15680      15690      15700      15710      15720
GCCTCCGCCG CCAGCCCCGC CTGCGCCTCC ACCGATGGTA GATTTATCAT CAGCTCCACC

     15730      15740      15750      15760      15770      15780
ACCGCCGCCA TTAGTAGATT TGCCGTCTGA AATGTTACCA CCGCCTGCAC CATCGCTTTC

     15790      15800      15810      15820      15830      15840
TAACGTGTTG TCTGAATTAA AATCGGGCAC AGTTAGATTG AAACCCGCCC AAAAACGCCC

     15850      15860      15870      15880      15890      15900
GCAATCAGAA ATAATTCCAA AAAGCTCAAC TACAAATTTG ATCGCGGACG TGTTAGCCGA

     15910      15920      15930      15940      15950      15960
CACAATTAAT AGGCGTCGTG TGGCTATGGC AAAATCGTCT TCGGAAGCAA CTTCTAACGA

     15970      15980      15990      16000      16010      16020
 AGGGTTGG GACGACGACG ATAATCGGCC TAATAAAGCT AACACGCCCG ATGTTAAATA

     16030      16040      16050      16060      16070      16080
TGTCCAAGCT ACTAGTGGTA CCGCTTGGCA GAACATATCC ATCGCGTCCG CCATCTCCAG

     16090      16100      16110      16120      16130      16140
CAGCCGCACG CGGCGCATCT CGGGCAGCGT TGGGTCCTGG CCACGGGTGC GCATGATCGT

     16150      16160      16170      16180      16190      16200
GCTCCTGTCG TTGAGGACCC GGCTAGGCTG GCGGGGTTGC CTTACTGGTT AGCAGAATGA

     16210      16220      16230      16240      16250      16260
ATCACCGATA CGCGAGCGAA CGTGAAGCGA CTGCTGCTGC AAAACGTCTG CGACCTGAGC

     16270      16280      16290      16300      16310      16320
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     16330      16340      16350      16360      16370      16380
 CCTGCACC ATTATGTTCC GGATCTGCAT CGCAGGATGC TGCTGGCTAC CCTGTGGAAC

     16390      16400      16410      16420      16430      16440
ACCTACATCT GTATTAACGA AGCGCTGGCA TTGACCCTGA GTGATTTTTC TCTGGTCCCG

     16450      16460      16470      16480      16490      16500
CCGCATCCAT ACCGCCAGTT GTTTACCCTC ACAACGTTCC AGTAACCGGG CATGTTCATC

     16510      16520      16530      16540      16550      16560
ATCAGTAACC CGTATCGTGA GCATCCTCTC TCGTTTCATC GGTATCATTA CCCCCATGAA

     16570      16580      16590      16600      16610      16620
CAGAAATCCC CCTTACACGG AGGCATCAGT GACCAAACAG GAAAAAACCG CCCTTAACAT

     16630      16640      16650      16660      16670      16680
GGCCCGCTTT ATCAGAAGCC AGACATTAAC GCTTCTGGAG AAACTCAACG AGCTGGACGC

     16690      16700      16710      16720      16730      16740
GGATGAACAG GCAGACATCT GTGAATCGCT TCACGACCAC GCTGATGAGC TTTACCGCAG

     16750      16760      16770      16780      16790      16800
CTGCCTCGCG CGTTTCGGTG ATGACGGTGA AAACCTCTGA CACATGCAGC TCCCGGAGAC

     16810      16820      16830      16840      16850      16860
GGTCACAGCT TGTCTGTAAG CGGATGCCGG GAGCAGACAA GCCCGTCAGG GCGCGTCAGC

     16870      16880      16890      16900      16910      16920
GGGTGTTGGC GGGTGTCGGG GCGCAGCCAT GACCCAGTCA CGTAGCGATA GCGGAGTGTA
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DNASIS
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     16930      16940      16950      16960      16970      16980
TACTGGCTTA ACTATGCGGC ATCAGAGCAG ATTGTACTGA GAGTGCACCA TATGCGGTGT

     16990      17000      17010      17020      17030      17040
GAAATACCGC ACAGATGCGT AAGGAGAAAA TACCGCATCA GGCGCTCTTC CGCTTCCTCG

     17050      17060      17070      17080      17090      17100
CTCACTGACT CGCTGCGCTC GGTCGTTCGG CTGCGGCGAG CGGTATCAGC TCACTCAAAG

     17110      17120      17130      17140      17150      17160
GCGGTAATAC GGTTATCCAC AGAATCAGGG GATAACGCAG GAAAGAACAT GTGAGCAAAA

     17170      17180      17190      17200      17210      17220
GGCCAGCAAA AGGCCAGGAA CCGTAAAAAG GCCGCGTTGC TGGCGTTTTT CCATAGGCTC

     17230      17240      17250      17260      17270      17280
CGCCCCCCTG ACGAGCATCA CAAAAATCGA CGCTCAAGTC AGAGGTGGCG AAACCCGACA

     17290      17300      17310      17320      17330      17340
GGACTATAAA GATACCAGGC GTTTCCCCCT GGAAGCTCCC TCGTGCGCTC TCCTGTTCCG

     17350      17360      17370      17380      17390      17400
ACCCTGCCGC TTACCGGATA CCTGTCCGCC TTTCTCCCTT CGGGAAGCGT GGCGCTTTCT

     17410      17420      17430      17440      17450      17460
CATAGCTCAC GCTGTAGGTA TCTCAGTTCG GTGTAGGTCG TTCGCTCCAA GCTGGGCTGT

     17470      17480      17490      17500      17510      17520
GTGCACGAAC CCCCCGTTCA GCCCGACCGC TGCGCCTTAT CCGGTAACTA TCGTCTTGAG

     17530      17540      17550      17560      17570      17580
TCCAACCCGG TAAGACACGA CTTATCGCCA CTGGCAGCAG CCACTGGTAA CAGGATTAGC

     17590      17600      17610      17620      17630      17640
AGAGCGAGGT ATGTAGGCGG TGCTACAGAG TTCTTGAAGT GGTGGCCTAA CTACGGCTAC

     17650      17660      17670      17680      17690      17700
ACTAGAAGGA CAGTATTTGG TATCTGCGCT CTGCTGAAGC CAGTTACCTT CGGAAAAAGA

     17710      17720      17730      17740      17750      17760
GTTGGTAGCT CTTGATCCGG CAAACAAACC ACCGCTGGTA GCGGTGGTTT TTTTGTTTGC

     17770      17780      17790      17800      17810      17820
AAGCAGCAGA TTACGCGCAG AAAAAAAGGA TCTCAAGAAG ATCCTTTGAT CTTTTCTACG

     17830      17840      17850      17860      17870      17880
GGGTCTGACG CTCAGTGGAA CGAAAACTCA CGTTAAGGGA TTTTGGTCAT GAGATTATCA

     17890      17900      17910      17920      17930      17940
AAAAGGATCT TCACCTAGAT CCTTTTAAAT TAAAAATGAA GTTTTAAATC AATCTAAAGT

     17950      17960      17970      17980      17990      18000
ATATATGAGT AAACTTGGTC TGACAGTTAC CAATGCTTAA TCAGTGAGGC ACCTATCTCA

     18010      18020      18030      18040      18050      18060
GCGATCTGTC TATTTCGTTC ATCCATAGTT GCCTGACTCC CCGTCGTGTA GATAACTACG

     18070      18080      18090      18100      18110      18120
ATACGGGAGG GCTTACCATC TGGCCCCAGT GCTGCAATGA TACCGCGAGA CCCACGCTCA

     18130      18140      18150      18160      18170      18180
CCGGCTCCAG ATTTATCAGC AATAAACCAG CCAGCCGGAA GGGCCGAGCG CAGAAGTGGT

     18190      18200      18210      18220      18230      18240
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DNASIS
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CCTGCAACTT TATCCGCCTC CATCCAGTCT ATTAATTGTT GCCGGGAAGC TAGAGTAAGT

     18250      18260      18270      18280      18290      18300
AGTTCGCCAG TTAATAGTTT GCGCAACGTT GTTGCCATTG CTGCAGGCAT CGTGGTGTCA

     18310      18320      18330      18340      18350      18360
CGCTCGTCGT TTGGTATGGC TTCATTCAGC TCCGGTTCCC AACGATCAAG GCGAGTTACA

     18370      18380      18390      18400      18410      18420
TGATCCCCCA TGTTGTGCAA AAAAGCGGTT AGCTCCTTCG GTCCTCCGAT CGTTGTCAGA

     18430      18440      18450      18460      18470      18480
AGTAAGTTGG CCGCAGTGTT ATCACTCATG GTTATGGCAG CACTGCATAA TTCTCTTACT

     18490      18500      18510      18520      18530      18540
GTCATGCCAT CCGTAAGATG CTTTTCTGTG ACTGGTGAGT ACTCAACCAA GTCATTCTGA

     18550      18560      18570      18580      18590      18600
GAATAGTGTA TGCGGCGACC GAGTTGCTCT TGCCCGGCGT CAACACGGGA TAATACCGCG

     18610      18620      18630      18640      18650      18660
CCACATAGCA GAACTTTAAA AGTGCTCATC ATTGGAAAAC GTTCTTCGGG GCGAAAACTC

     18670      18680      18690      18700      18710      18720
TCAAGGATCT TACCGCTGTT GAGATCCAGT TCGATGTAAC CCACTCGTGC ACCCAACTGA

     18730      18740      18750      18760      18770      18780
TCTTCAGCAT CTTTTACTTT CACCAGCGTT TCTGGGTGAG CAAAAACAGG AAGGCAAAAT

     18790      18800      18810      18820      18830      18840
GCCGCAAAAA AGGGAATAAG GGCGACACGG AAATGTTGAA TACTCATACT CTTCCTTTTT

     18850      18860      18870      18880      18890      18900
CAATATTATT GAAGCATTTA TCAGGGTTAT TGTCTCATGA GCGGATACAT ATTTGAATGT

     18910      18920      18930      18940      18950      18960
ATTTAGAAAA ATAAACAAAT AGGGGTTCCG CGCACATTTC CCCGAAAAGT GCCACCTGAC

     18970      18980      18990      19000      19010      19020
GTCTAAGAAA CCATTATTAT CATGACATTA ACCTATAAAA ATAGGCGTAT CACGAGGCCC

     19030      19040      19050      19060      19070      19080
TTTCGTCTTC AAGAA..... .......... .......... .......... ..........
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/03935

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/90 C12N15/85 C12Q1/68 C12N5/10 C12N9/12
C12N15/13 C07K16/28 C12N15/12 C07K14/705 G01N33/53
C12N15/62 C07K19/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 94 11523 A (IDEC PHARMACEUTICALS CORPORATION (US); REFF MITCHELL E. (US)) 26 May 1994 cited in the application see abstract see page 9, line 21 - page 10, line 29 see page 41, line 19 - page 42, line 19; figure 6 ---	1,4-8, 11,12, 25-29, 31,32
A	US 5 464 764 A (CAPECCHI MARIO R. AND KIRK THOMAS R.) 7 November 1995 see abstract see column 13, line 32 - column 14, line 5 ---	1
A	WO 94 05784 A (UNITED STATES AMERICA REPRESENTED BY THE SECRETARY US DPT. AGRICULTURE) 17 March 1994 see abstract ---	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publicationdate of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of theinternational search
23 July 1998

Date of mailing of the international search report
05/08/1998

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1

International Application No
PCT/US 98/03935

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 93 24642 A (TSI CORPORATION (US)) 9 December 1993 see abstract ---	1
A	BARNETT R.S. ET AL.: "Antibody production in chinese hamster ovary cells using an impaired selectable marker" ACS SYMPOSIUM SERIES: ANTIBODY EXPRESSION AND ENGINEERING, vol. 604, 1995, pages 27-40, XP002072464 -----	

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I. ational Application No
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		AU 5613294 A	08-06-1994
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		DE 669986 T	10-10-1996
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		MX 9305183 A	31-05-1994
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